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GROWTH

A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

VOLUME XIX, NUMBER 2

PAPERS 467-471

JUNE, 1955 **$6.00 A YEAR**

Entered as second class matter on October 20, 1941, at the Post Office,

GROWTH

A journal for the coordination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a cooperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy, and anthropology; just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamins, and hormones.

It is published four times a year during March, June, September and December at $6.00 per volume (outside U. S. and Canada, $6.50). Single numbers $2.00. There will be one volume each year.

All manuscripts and subscriptions should be sent to: The Editors of Growth, Lankenau Hospital Research Institute, Fox Chase, Philadelphia 11, Pa.

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Growth, 1955, **19**, 45-56.

EFFECT OF BIOTIN DEFICIENCY ON BODY AND ORGAN GROWTH AND ACCESSORY REPRODUCTIVE STRUCTURES IN MALE RATS

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(Received for publication April 26, 1955)

Although a considerable body of literature relating to the biotin deficiency syndrome in rats is available (Hertz, 1946), certain aspects remain to be considered. For example, organ growth in biotin-deficient rats demands attention and the influence of reduced food intake upon the body and organ weights in the animals fed with special diets requires examination. This communication attempts to supply such information by comparing the growth patterns of animals on special diets with others receiving complete rations or those subjected to partial inanition. Evidence regarding lesions characteristically found in the testes of the biotin-deficient animals is also included.

MATERIALS AND METHODS

A total of 73 male albino rats of a highly inbred strain (Wistar lineage) was employed. These were dispersed into 9 groups (Table 1). Groups I, II and VI received a standard laboratory diet (Purina laboratory chow). In groups II, III, IV, VII and VIII, sulfaguanidine (½-1%) was incorporated into the rations. In groups III and IV a purified diet deficient in biotin made according to the method of Welch and Wright (1943) was offered and in addition the rats in group IV were injected with 30 ug. of biotin on alternate days commencing with the first day on the diet. The animals in groups V and IX were placed on semi-starvation rations: those in group V were restricted initially to 4 grams of complete diet per day and subsequently this amount was increased to 5 and then 6 grams per day while the rats in group IX were continued on 4 grams per day throughout. Caloric intake was restricted in these two groups so as to ap-

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proximate the level ingested by the egg white-dieted animals and, thus, to provide a basis for assessing the contribution of starvation in the latter animals. The animals in groups VII and VIII were fed a diet prepared by thoroughly mixing the finely divided standard ration with 60% spray dried egg white. (Liberal amounts of egg white were supplied through the courtesy of H. M. Slosberg, Henningsen-Lamesa Inc.) Biotin in doses of 30 ug. every other day was administered to the rats in group VIII.

All rats were at the weanling stage when placed on the respective diets and littermates were evenly distributed throughout the groups insofar as possible. Each animal was isolated in a nutrition cage on a wire screen to prevent coprophagy. Weighed amounts of the respective food were available *ad lib.* to all animals except those on restriction. Fresh water was present at all times. Records of body weights and food intake were made twice a week.

Termination of the experiments depended upon the appearance of the deficiency symptoms. By the 60th day the rats in group VII exhibited signs of pronounced biotin deficiency. Therefore these animals together with those in groups VI, VIII, and IX were sacrificed at that time. The animals in groups I-V were terminated at 100 days. From each animal the following organs were carefully excised, weighed to the nearest milligram on a precision balance and placed into Bouin's picro-formol fixative: pituitary (entire), thyroid (paired lobes), adrenal (both glands), testis (1), seminal vesicle (1), ventral prostate, thymus and spleen. Sections of 4-6 micra were made of all tissues. These were then stained in Harris' hematoxylin and counterstained with eosin and all were studied histologically.

Evaluation of the weight data included obtaining the means, the standard deviations and the standard error of the means for each group of animals. For levels of significance t-ratios (Fisher, 1934) were employed. Using Fisher's formula comparisons were made between the groups I-V and between the groups VI-IX.

Observations and Results

The untreated egg white rationed animals (group VII) began to exhibit symptoms of biotin deficiency (red scaly mouth, blepharitis, spectacle eye) at the fifth week. At sacrifice kangaroo posture was noticeable in some of these rats. By contrast the rats in group VIII

which also ingested the egg white rations but which received biotin therapy were asymptomatic as were those in the remaining groups.

Table 1 presents the data regarding body weights and organ weights in terms of 100 grams of body weight. Figure one graphically depicts the body weight responses of the various groups. The im-

Fig. 1 BODY WEIGHTS

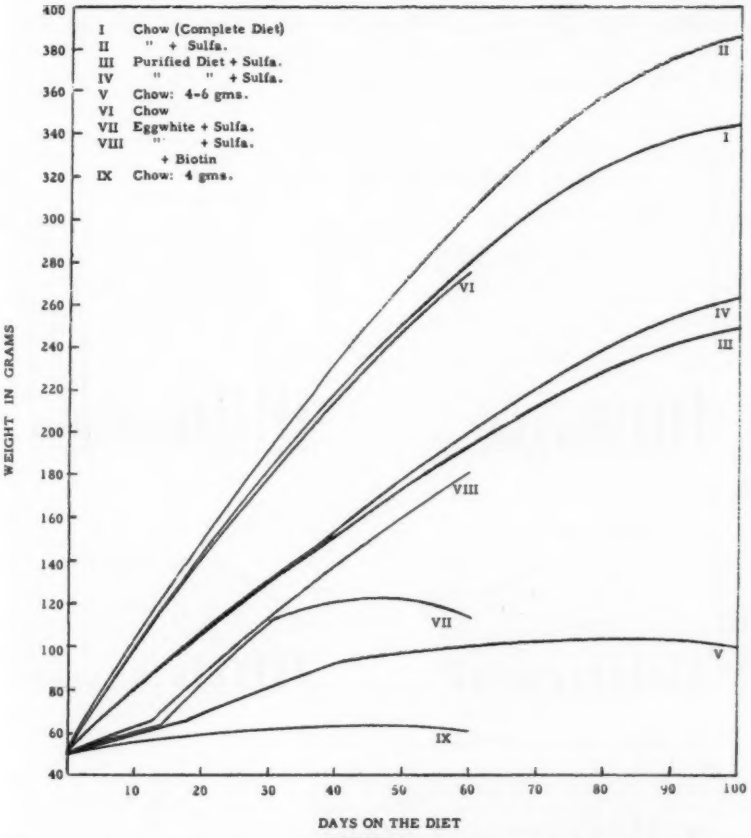
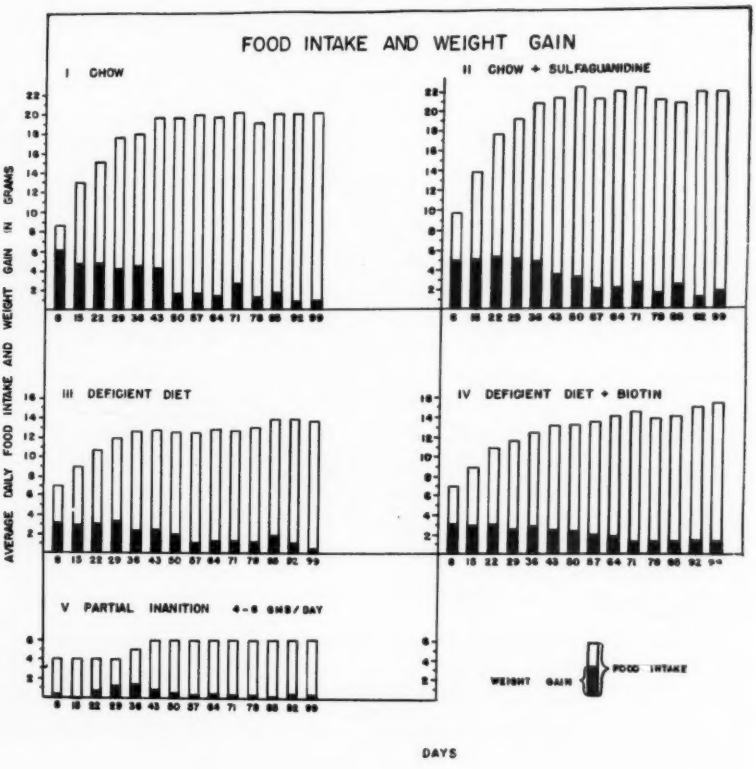


FIGURE 1

Comparing curve VIII to curve VII indicates that biotin is of definite growth promoting value.

proved growth of the rats ingesting the complete ration plus sulpha compared with those which ate the unadulterated chow confirms previous observations (Spector, 1948). The difference in weights of the two groups of animals (III and IV) fed the purified diet is not significant and biotin appeared to have no great growth promoting influence in the group so treated. Biotin was of definite growth promoting value in the group of rats which received injections of this vitamin while eating the egg white rations. Restriction of food intake suppressed the body weight curves of such starved animals cor-

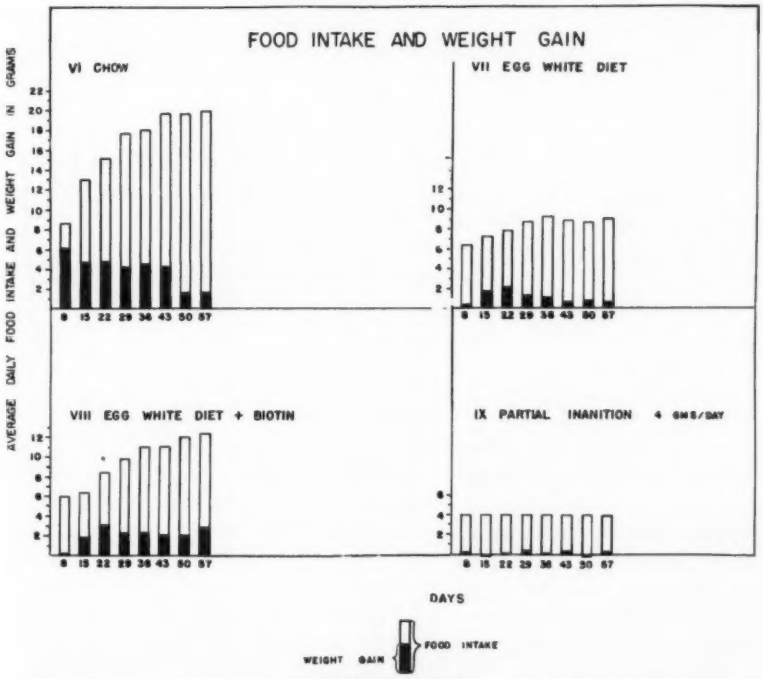
FIG. 2



respondingly. Food intake and weight data are summarized in the bar graphs of Figures 2 and 3. Of significance are the measurements relating the inanition groups to the experimental series. An allotment of 4 grams of complete diet per day eaten by the rats in group IX corresponds almost precisely to the amount of this food ingested by the untreated egg white group considering that 60% of the latter diet consisted of egg white while the remainder was the standard laboratory ration. By the same token, an allotment of 5 grams of food per day permitted the rats in group V closely approximates the amount of complete diet consumed by the treated egg white group.

With reference to the organ weights, the outstanding features which

FIG. 3


FOOD INTAKE AND WEIGHT GAIN
VI CHOW
VII EGG WHITE DIET
VIII EGG WHITE DIET + BIOTIN
IX PARTIAL INANITION 4 GMS/DAY
AVERAGE DAILY FOOD INTAKE AND WEIGHT GAIN IN GRAMS
DAYS
WEIGHT GAIN
FOOD INTAKE

FIGURES 2 AND 3

These graphs indicate the effect of the various diets upon the efficiency of utilization of the respective food.

TABLE 1
Body and Organ Weights in Control and Biotin-Deficient Rats

Group	Rats		Body Weight (gms.) Mean + S.D.		Organ Weights (mg./100 gms. wt.) Mean + S.E.							
			Initial	Final	Pituitary	Thyroid	Adrenals	Testis	Seminal Vesicle	Prostate	Thymus	Spleen
I	8	Chow (complete diet)	47 ± 6	346 ± 37	3.26 ± .14	8.65 ± .45	18.3 ± 1.6	518 ± 17	113 ± 6	146 ± 6	92 ± 4	150 ± 4
II	8	Chow plus sulfa	47 ± 5	388 ± 43	2.97 ± .21	9.47 ± .71	16.7 ± 1.0	483 ± 18	123 ± 11	154 ± 15	86 ± 4	149 ± 10
III	8	Purified + sulfa	48 ± 3	249 ± 29	3.33 ± .17	11.50 ± .75	15.8 ± 1.8	415 ± 29	105 ± 10	129 ± 9	95 ± 4	167 ± 14
IV	8	Purified + sulfa + biotin	44 ± 7	265 ± 19	3.29 ± .20	10.90 ± .75	18.4 ± 3.0	343 ± 35	129 ± 8	139 ± 11	118 ± 13	165 ± 6
V	8	Chow: 4-6 gms.	46 ± 5	100 ± 11	4.02 ± .33	15.44 ± 2.0	30.8 ± 3.0	467 ± 24	52 ± 4	42 ± 8	86 ± 6	125 ± 10
VI	8	Chow	46 ± 7	280 ± 19	3.38 ± .24	11.10 ± .90	18.5 ± .7	609 ± 15	135 ± 21	141 ± 19	118 ± 22	214 ± 8
VII	10	Egg white-chow + sulfa	47 ± 2	114 ± 38	4.14 ± .37	17.20 ± 1.6	31.8 ± 3.0	477 ± 18	17 ± 2	18 ± 2	73 ± 12	166 ± 10
VIII	10	Egg white-chow + sulfa + biotin	45 ± 6	176 ± 39	3.62 ± .22	17.10 ± 1.9	24.3 ± 1.2	739 ± 18	129 ± 16	117 ± 11	116 ± 7	194 ± 11
IX	5	Chow: 4 gms.	44 ± 4	63 ± 7	3.87 ± .37	15.8 ± 1.7	40.1 ± 2.9	234 ± 3	7 ± 2	10 ± 2	50 ± 6	154 ± 15

are of interest here are the values relating to the accessory organs of reproduction. Atrophic prostates and seminal vesicles were found in the two inanition groups and the untreated egg white rationed animals whereas the weights of these organs in the biotin injected egg white-dieted rats were not different from those of the remaining groups which were in the normal range. The adrenal weight-body weight ratio was elevated in the two inanition groups and the untreated egg white rationed rats. The indication that increased adrenal weight reflects increased activity of the suprarenals is also found in the decreased thymic weights of these groups. When observed weights are compared, the thymolytic effect of the increased adrenal activity is emphasized.

The histological findings confirmed the weight observations in the main and offered additional valuable information. The testes and accessory organs of reproduction in the animals in groups I, II and VI (chow fed, with or without sulfa) were normal (Fig. 1-3). In contrast the gonads of the egg white fed rats which were not given supplementary biotin were strikingly aberrant. The main pathology was most frequently found to be a lack of germinal elements (Fig. 4) so that only a basal rim of spermatogonia and sustentacular cells remained. In those cases where depletion had not proceeded to such an extreme, spermatogenesis seemed to have been arrested in the late primary or early secondary spermatocyte stage. The atrophic character of the seminal vesicles and ventral prostates of these animals (Fig. 5 and 6) which resembled the castrate condition was obvious. In sharp distinction were the respective organs of the rats fed egg white diet but which were injected with biotin; these organs were microscopically indistinguishable from the normal (Fig. 7-9). The testes of the rats receiving limited amounts of food resembled those of the immature rat (Fig. 10): seminiferous tubules were uniformly small and closely packed; no lumina or spermatids were seen. The accessory organs of reproduction of these animals were atrophic and devoid of secretory activity (Fig. 5 and 6). The gonads of the purified dieted animals were comprised of shrunken and distorted tubules in which the germinal elements were in disarray. The outstanding feature was the presence of giant multinucleate cells, appearing as beads (Fig. 11) in such abundance as to call immediate attention to the avitaminosis E syndrome. Interstitial tissue was prominent and its functional capacity unaltered as evidence by the weights of the accessory reproductive organs and their normal histology.

FIGURE 1
Normal testis, germinal epithelium in all stages of differentiation.

FIGURE 2
Normal seminal vesicular epithelium is tall columnar.

FIGURE 3
Normal prostatic epithelium is columnar and light areas (or halos) are seen in the supranuclear region.

FIGURE 4
Untreated egg white fed rat's testis have seminiferous tubules of small diameter and few germinal elements are found.

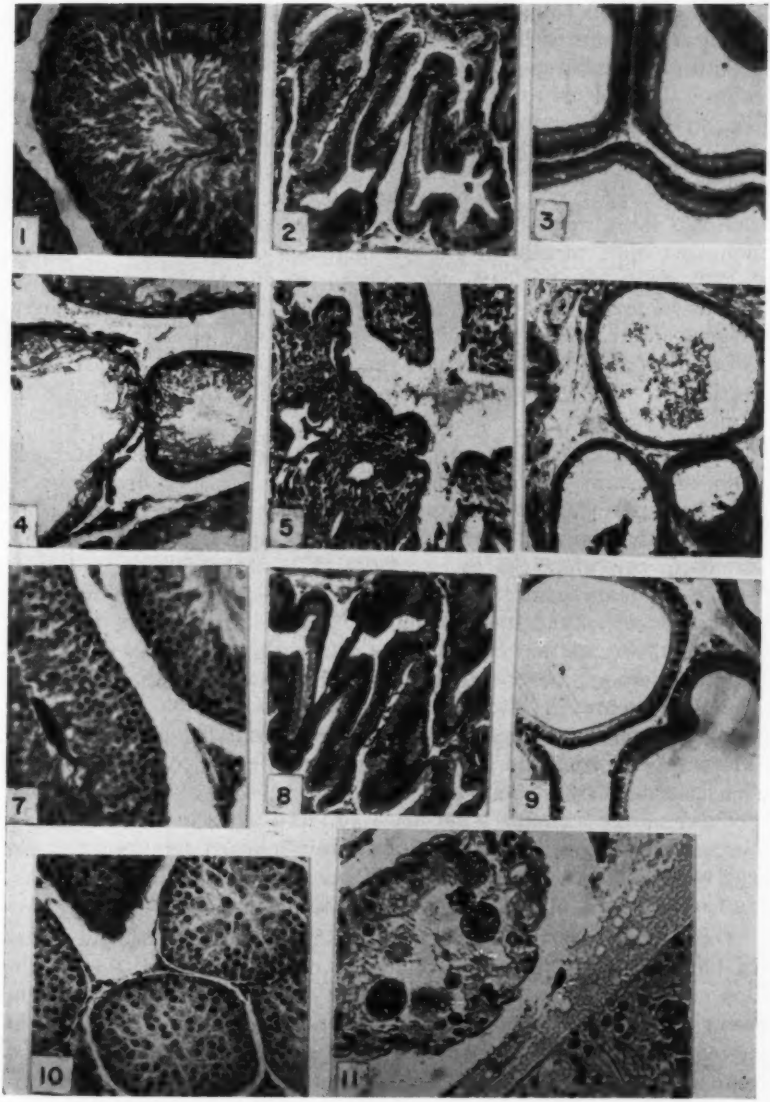
FIGURE 5
The seminal vesicles of the egg white dieted rat show inactive epithelium, little evidence of secretion and debris in the lumina.

FIGURE 6
The prostates of starved rats are atrophic.

FIGURES 7-9
Biotin injected into rats ingesting egg white rations prevents the lesions noted in the testis (7), seminal vesicles (8) and prostate (9) of rats consuming egg white without biotin therapy.

FIGURE 10
The testis of the starved rat represents a condition of immaturity and is unlike that of the untreated egg white fed rat.

FIGURE 11
Animals fed a purified diet possessed testes which gave evidence of vitamin E deficiency (note the large multinucleate cells). Their accessory organs were normal.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No striking histological pathology other than adrenal cortical hypertrophy (in confirmation of the groups exhibiting elevated adrenal weights) was apparent.

Discussion

The characteristic symptomatology of biotin deficiency as first described by Boas (1927) was found in the group of rats which ingested the egg white rations without benefit of biotin therapy. In such rats body weight and seminal vesicle and prostate weights were depressed while adrenal weight was increased. Some measure of these growth responses may have been due to the effects of inanition since starvation groups exhibited similar responses. However, the body weights of the untreated egg white fed rats were clearly superior to those of either group of starved rats, thus it would seem that some portion of the body weight depression is a function of the lack of available biotin.

The testicular lesions noted in the untreated egg white animals cannot be ascribed to reduced caloric intake since such findings were not obtained in animals whose food intake was severely restricted. The fact that injected biotin prevented the appearance of testicular lesions in egg white fed rats suggests that this vitamin may play a role in the maintenance of the gonads. The condition of immaturity in the gonads of the starved animals confirms the findings of Siperstein (1920) and the atrophic character of the accessory organs of reproduction appears to result from the condition termed pseudohypophysectomy by Mulinos and Pomerantz (1940). Biotin prevented atrophy of the accessory organs in the rats ingesting egg white. The intake of complete diet by such animals corresponded closely to that consumed by the rats permitted only 5 grams of standard ration per day. Since atrophic accessory reproductive organs were found in the semi-starved rats and not in the biotin-injected rats forced to eat egg white rations, it is possible that the vitamins facilitated the release and/or synthesis of gonadotrophic hormones.

Okey, Pencharz and Lepkovsky (1950) indicated that the testes of biotin-deficient rats were small but otherwise normal, yet in the absence of microscopic examination it is easily understood that on the basis of weight alone one might be led to such a conclusion. The testes of rats which ingested the purified rations were affected in a manner precisely as described by Mason (1933) as being specific in

avitaminosis E and it appears clear that this aberration was due to lack of this vitamin. The fact that biotin had no corrective action here is support for this conclusion. Moreover, although the purified rations were deficient in biotin, they were not completely lacking this factor and the amount available to these animals was found to be sufficient to prevent a biotin deficiency state (Litsky, *et al.*, 1953). In addition, since the accessory organs of the purified rationed rats were normal, the condition is entirely different from that observed in egg white dieted rats.

When viewed in the overall aspect, the current results indicate the salutary effects of biotin upon the body and organ growth patterns in the male rats in the specific deficiency state with which it is associated. Such observations imply a vitamin-hormonal relationship which may also be integrated with enzymatic function in view of the important role played by biotin in several enzyme systems (Lichstein, 1951). Any study providing evidence of such far-reaching implications is instructive and might provide impetus for further examination.

Summary

Weanling male rats were fed standard laboratory rations, purified diets, restricted amounts of food or rations rich in egg white. Body weights and food consumption were recorded. At sacrifice the weights of the pituitaries, thyroids, adrenals, seminal vesicles, prostates, thymi and spleens were taken. Biotin therapy prevented symptomatology and encouraged maintenance of body and organ weights in the condition normally leading to biotin deficiency when rats ingested egg white enriched diets. Also, testicular lesions which were found in untreated biotin-deficient rats were not present in similarly fed animals receiving biotin. It appears that although some of the body weight suppression and organ weight change in the deficiency state may be attributable to reduced food intake, certain features, notably testicular lesions, cannot be so ascribed.

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Growth, 1955, **19**, 57-74.

POSTNATAL CHANGES IN PROTEOLYTIC ACTIVITY AND IN THE MORPHOLOGY OF THE SUBMAXILLARY GLAND IN MALE AND FEMALE ALBINO RATS

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(Received for publication April 26, 1955)

Sex differences in salivary glands were first described for the submaxillary glands of mice (Lacassagne, '40a). This unexpected finding of a secondary sex difference in a secretory organ has since then been confirmed by numerous investigators (Lacassagne, '40b; Fekete, '41; Causse and Lacassagne, '42; Chaulin-Serviniere, '42; Raynaud, '44; Junqueira, '49; and Arvy and Gabe, '50). These authors found that the gland of the adult male mouse showed a preponderance of intralobular ducts over acini. The cells of these ducts in male animals were tall columnar, their nuclei were located at the base, and their cytoplasm was almost completely filled with large, spherical secretion granules. In the female, the relative area occupied by the intralobular ducts was smaller; it was approximately the same as that occupied by acini. The cells making up the ducts in the female were of low columnar shape or cuboidal and contained fewer cytoplasmic granules than those of the male. A number of quantitative studies were made which confirmed the larger diameter and larger relative area occupied by the intralobular ducts in the male.

Castration of male animals caused the submaxillary gland to atrophy and assume the morphologic appearance of the female gland. Conversely, the glands of female mice showed enlarged ducts and greatly increased numbers of secretory granules after administration of male sex hormones (Lacassagne, '40b; and Raynaud, '44). These experiments seem to make a case of a secretory function under

This investigation was carried out under Contract DA-49-007-MD662 with the Medical Research and Development Board, Office of the Surgeon General of the United States Army.

the control of a hormone. The nature of the secretion was not known until Junqueira and his associates (Fajer, Rabinovitch, and Frankenthal, '49) showed that extracts of mice submaxillary glands had protease activity, as seen by the liberation of tyrosine from casein. This was found to be very slight before puberty and of the same degree in both sexes. In adult males, however, it was 36 times as high and in adult females 19 times as high as before puberty. Although he did not obtain direct evidence, Junqueira concluded that a proteolytic enzyme was contained in the secretory granules of the intralobular ducts.

In many species of rodents, the intralobular ducts of the submaxillary gland are prominent and contain cells of secretory appearance. Sex differences have not been observed in the glands, however. Studies in the rat showed that in this species the submaxillary glands are morphologically alike in the two sexes. Careful quantitative studies showed that at most the diameters of the intralobular ducts are slightly larger in the male (Grad and LeBlond, '49). The ratio of ducts to acini in the adult male rat was determined by Arvy, Debray and Gabe ('50), but the female has not been studied. The ratio appears to be lower in the rat than in the mouse. The slight differences seen between adult male and female rats were found absent before puberty. The diameters of the intralobular ducts (Grad and LeBlond, '49) were found to double their length between the ages of 10 and 150 days, the growth curves being closely alike in male and female animals. Experiments with castration and administration of sex hormones have given uncertain results.

Rothschild and Junqueira ('51), using globin as a substrate, have shown that there is protein breakdown in the submaxillary glands of females, but no quantitative study or comparison between male and female and young and adult rats has been made. A study in this laboratory has confirmed the presence of proteolytic activity in the submaxillary glands of rats. The enzyme was found to be a relatively unspecific protease resembling trypsin, capable of degrading casein, gelatin and benzoyl-arginine-amide. The optimal pH was 9.6. Enzyme activity was found to be of a rather high order, comparable to that of the pancreas. Only traces of activity were seen in the other salivary glands. Preliminary experiments showed that the amount of breakdown was greater in males than in females (Sreebny, '53a).

The present report deals with the changes in proteolytic activity

and in the morphology of the submaxillary gland in male and female animals during postnatal development.

Methods and Materials

Five developmental stages were studied: early and late prepuberal rats, or 15 and 30 days of age; early and late puberty, or 60 and 90 days of age; and young adult animals, or 150 days of age. In male and female animals of these ages, the submaxillary gland was examined histologically and from a quantitative morphological point of view, and an assay of its proteolytic activity was made.

The animals were of the Sprague-Dawley strain. A total of 59 rats were used for the study reported in this section. There were 5 to 7 male and the same number of female animals in each age group. The submaxillary gland of the left side was used for chemical determinations, that of the right for histological study.

The animals were killed by ether, and the capsules containing the submaxillary and sublingual glands[1] were dissected free of the adjacent organs, placed in liquid air and then transferred to a Gersh-Altmann freeze-drying unit for lyophilization.

An extract was made by grinding the finely cut frozen-dried gland in a mortar and slowly adding saline or buffer solutions in amounts sufficient for the concentration of the extracts to be 7 mg. of dry weight per ml. Protease activity was determined, using azocoll as a substrate, as described in a previous report (Sreebny, Meyer and Bachem, '54). The following experimental specifications were used: 3 mg. of azocoll, 1 ml. of gland extract, 3 ml. of pH 9.6 phosphate buffer, and an incubation period of 40 minutes at 37°C. The amount of protein breakdown during this period was determined as the optic density of the filtrate.

On varying the quantity of gland tissue incubated with a constant quantity of substrate, it had been found that during a 40 minute period the optic density varied with the square root of the amount of tissue extract (Sreebny, Meyer and Bachem, '54). This relation was used to compute the enzyme content of each gland sample. The value unity was assigned to the average of the 30 day old males, and all other enzyme concentrations were expressed as multiples of this figure. The 30 day old male animals were chosen as the standard

[1] Both glands were used for chemical determinations. The sublingual gland weighs about a fifth of the submaxillary. Under the conditions used for assay it does not have proteolytic activity.

because in this group the figures for optic density showed a smaller standard deviation than in any other.

FINDINGS

1. *Morphological findings*

Between the ages of 15 and 150 days, the glands grow from about 20 to 100 mg. in the male and to about 65 mg. in the female (figure 1). In both sexes, the growth curve of the gland resembles that of body weight.

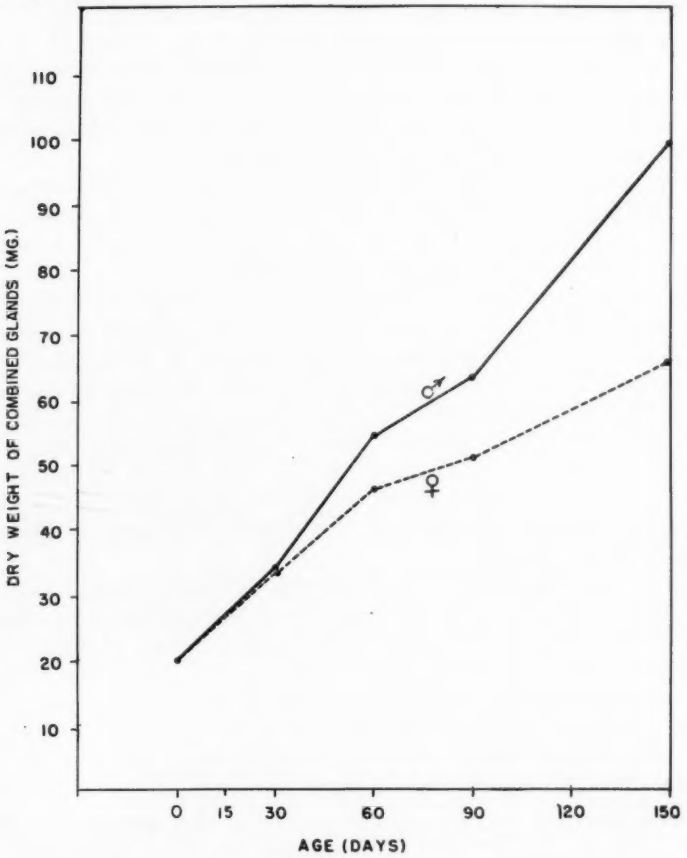

FIGURE 1
Dry weights of the combined glands with age.

The general morphologic pattern remains the same in all stages. Increase in size of gland occurs by increase in the number of parenchymatous elements due to proliferation of duct cells and their differentiation into either alveoli or into mature intralobular duct cells.

Duct cell buds differentiating into acini undergo a brief process of maturing into fully differentiated acinar cells. No transitional stages between the newly formed and the mature alveolus are encountered; some secretion granules are present from the youngest stage on. The number of alveoli increases until about 2 months of age. Maturation of the acinar portion of the gland runs ahead of that of the tubular portion. It is completed by 90 days of age. From 60 days on, the acini seem to lose ground and the intralobular portion to occupy an increasingly larger fraction of the gland.

The cells of the intralobular ducts which do not differentiate into acini undergo a slow transformation into secretory cells. This process occupies the whole age span studied. At the later stages, cells which are well advanced in the assumption of secretory characteristics are intermixed with others which still have the traits of younger stages. At 60 days, some of the nuclei of the intralobular duct cells are flattened and lie close to the periphery. In these cells the granules have grown in size and number. At 90 days, cells with round nuclei, few granules and distinct basal striation are still encountered adjacent to cells which contain large numbers of secretory granules with flattened nuclei and indistinct mitochondria. At 150 days, the ducts are a mixture of the advanced cell type seen at 90 days and of cells with signs of maximal secretory activity, in which the nucleus is flat and pressed toward the periphery of the cell and the cytoplasm entirely filled with coarse spherical eosinophilic stained secretion granules. (Figures 2 and 3). Secretion granules are occasionally seen in the duct lumen. Differences between the glands of male and female animals were not visible at any stage.

Age Changes in the Relative Amount of Intralobular Duct Tissue

On microscopic inspection it appeared that at successive stages an increasing fraction of the gland was occupied by cells of the intralobular ducts. Counts showed that in 1080 squares examined for each gland the number occupied by intralobular duct tissue varied as follows with increasing age (table 1):

TABLE 1
Quantitative Changes in the Morphologic Elements of the Submaxillary Gland with Age.

Age (Days)	Average Body Weight (Gm)		Dry Weight Glands (Mg)		Area of Ducts (in 1080)	
	Male	Female	Male	Female	Male	Female
15	42	45	19.7	20.6	180	250
30	108	98	33.7	33.0	216	250
60	195	146	54.1	46.3	297	302
90	253	187	63.0	50.8	340	351
150	522	261	99.0	65.6	430	453

The counts thus confirmed the impression of a progressive increase in the amount of duct tissue per field. They also confirmed the impression gained on inspection that the intralobular ducts in the glands of male and female animals at every one of the stages studied occupied the same fractions of the cross sectional area.

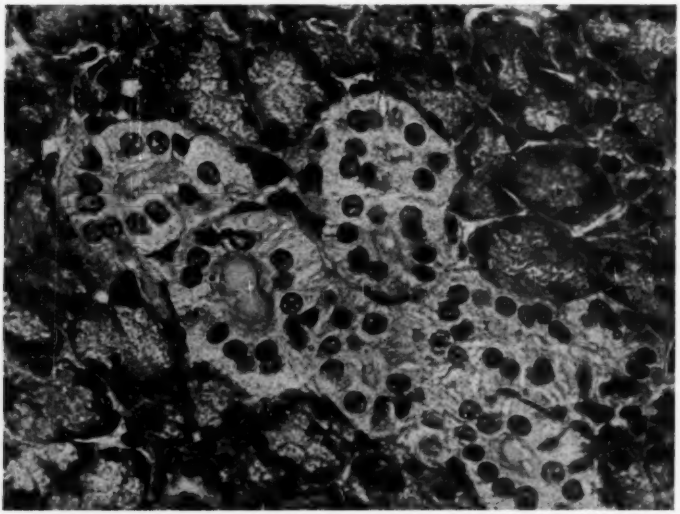

FIGURE 2
Section from the submaxillary gland of a 15-day old male rat. x660

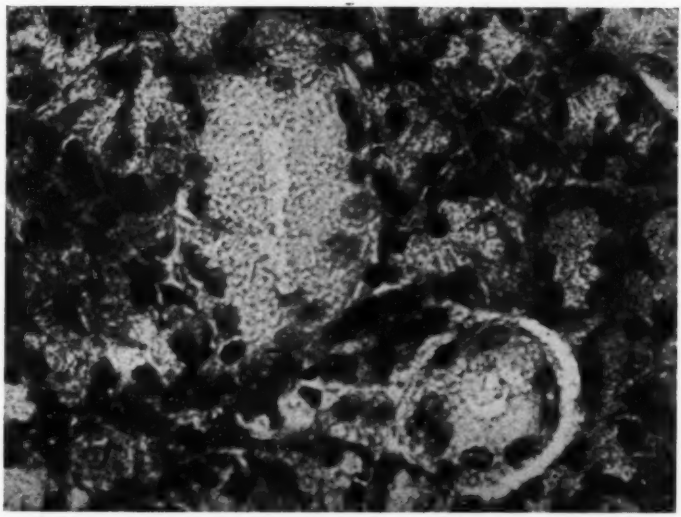

FIGURE 3
Section from the submaxillary gland of a 150-day old male rat. x660

2. *Proteolytic activity at different ages*

Enzyme activities per unit dry weight of submaxillary gland are given in table 2 and in figure 4. The total range of concentrations varied from 0.87 to 47.4 units.

In both male and female animals, the concentrations were stationary between 15 and 30 days, and there were progressively larger increases in the subsequent intervals. Concentrations in male animals increased forty-seven-fold over the 30 day values; those in female animals a little over twenty-fold.

The degree of proteolytic activity in male animals was higher than that in females from 60 days on. The difference was maximal at 90 days, when the proteolytic activity of male animals was about five times as high as that of the female ones. At 150 days, it was a little more than twice that of the females.

The differences in concentration between successive age groups and between male and female animals are listed in table 2. Statistical analysis of the group data showed that the changes in successive age

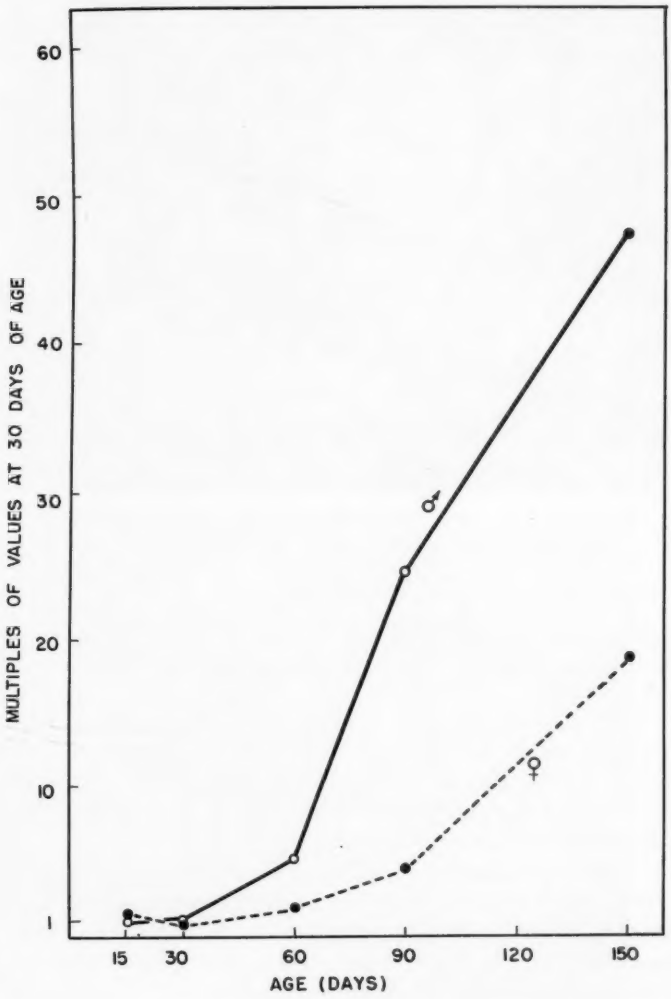

FIGURE 4
Protease activity of combined glands in rats of different ages.

groups are highly significant for the male animals. The female age sequence of differences in concentration is not established except for the change between 30 and 60 days.

TABLE 2
Changes in the Proteolytic Activity of the Glands of Rats

Sex	Age (Days)	Number of Animals	Average Azocoll Activity (O.D.)	Average Relative Concentrations (R)	Standard Deviation of R (σ)	Standard Error of R (σ_x)
Male	15	6	.032	1.15	0.33	0.13
Male	30	6	.030	1.00	0.10	0.04
Male	60	6	.065	5.01	2.76	1.17
Male	90	6	.141	24.50	9.51	3.88
Male	150	7	.203	47.40	12.49	4.74
Female	15	5	.034	1.34	0.45	0.20
Female	30	6	.028	0.87	0.15	0.06
Female	60	6	.044	2.12	0.63	0.25
Female	90	5	.062	4.66	2.68	1.20
Female	150	6	.118	18.99	16.94	7.33

Male and female animals have a rather narrow range of individual concentrations at 15 and 30 days, and wider scatter at older ages. The widest range of scatter occurs among the adult females. The differences in concentration between male and female animals were statistically significant for the 60-day old animals and the subsequent age groups, despite the wider scatter among the females.

Discussion

1. *Site of Proteolytic Activity*

The submaxillary glands of rats, like those of mice, show two distinct kinds of secretory cells, those of the acinar portion and the secretory cells of the intralobular ducts. At both these sites, increase in the number of secretory cells and increase in the number of granules in the cells occur between 15 and 150 days of age. From 60 days on, the increase in cell number and granule content is far more conspicuous and rapid in the intralobular cells while there is very little change in the acinar portion. Quantitative estimates show that the percentage of intralobular secretory cells per unit area increases in each successive age group and doubles between 30 and 150 days of age, at the expense of that occupied by acinar cells. There

is no reason to assume that sections in other planes would have shown different percentages of increase, because the morphology of the submaxillary gland is quite uniform. A volume per cent increase may therefore be estimated from the figures of increase in one plane according to the function $v = (\sqrt{a})^3$. So computed the volume increase in intralobular secretory cells is from 680 of 6,480 units at 30 days of age to 1,770 of 6,480 at 150 days of age for male animals, and of the same order for female animals. The acini and stroma decrease in proportion to the increase of the intralobular cells.

Since the proteolytic activity per unit volume increases with each successive age group, the present findings for the rat lead to the same tentative conclusion that Junqueira reached for mice, that is, that there is a high degree of proteolytic activity in postpuberal submaxillary glands and that the probable site of proteolytic activity is in the intralobular secretory cells.

2. *Relation between increase in proteolytic activity and increase in granules*

Both the specific proteolytic activity and the percentage volume occupied by granule-containing cells of the ducts increase with each successive age group. The increase in proteolytic activity, however, is far steeper than the increase in the volume percentage of tissue occupied by intralobular secreting cells. This does not disprove the tentative conclusion that these cells rather than the alveoli contain the proteolytic enzyme of the gland but it makes it necessary to assume that the secretory capacity of the cells, that is, number, size or enzyme content of the granules in the intralobular duct cells, increases to the extent to which the increase in the volume per cent of these cells fails to allow for the increase in specific proteolytic activity.

When the specific proteolytic activity and the volume percentage of intralobular tissue are plotted as the multiples of their values in 30-day old animals, the two curves are seen to diverge progressively (figures 5 and 6). The rate of increase of the two functions differs maximally during the interval 60-90 days, when tissue increase is slowed and proteolytic increase speeded up. The third curve on the graph is obtained when the relative enzyme concentrations are divided by the volume percentages of duct tissue. It represents the hypothetical increase in the size, number, or enzyme content of the

secretion granules. In the male animals, the highest rate of increase in granular capacity must have occurred between 60 and 90 days of age, and steady, much lower rates during the other intervals.

In female animals, the two curves of specific proteolytic activity and of specific amounts of intralobular tissue diverge much less. This is due to the lower rates of increase of proteolytic activity, the in-

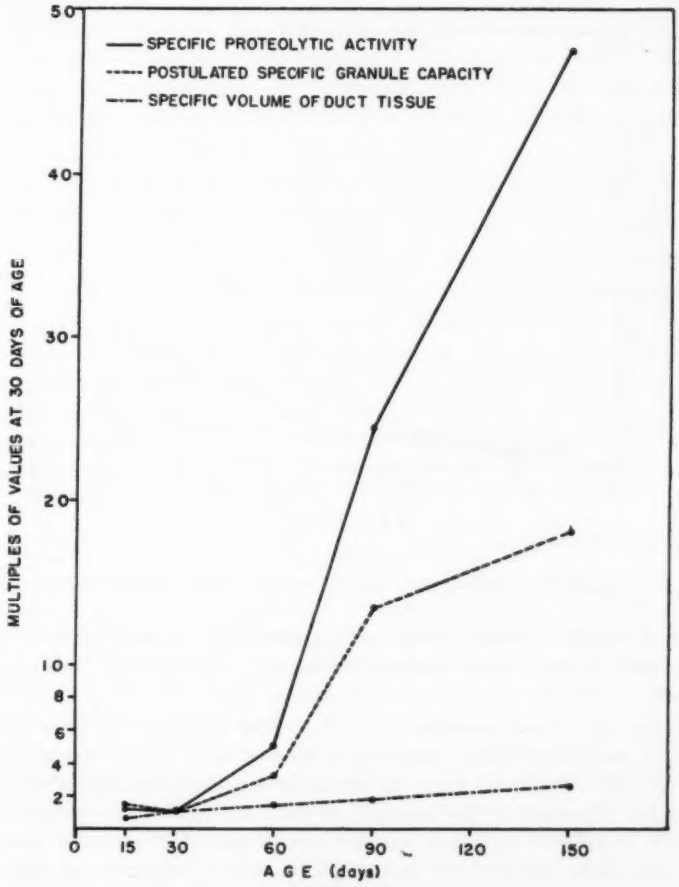

FIGURE 5

Specific proteolytic activity, granule capacity and duct mass with age (male rats).

crease in duct tissue being much the same in the two sexes. The curves show that a three-fold increase in granular capacity would have been necessary in order to account for the increase in chemical activity between 30 and 60 days. To account for the total increase be-

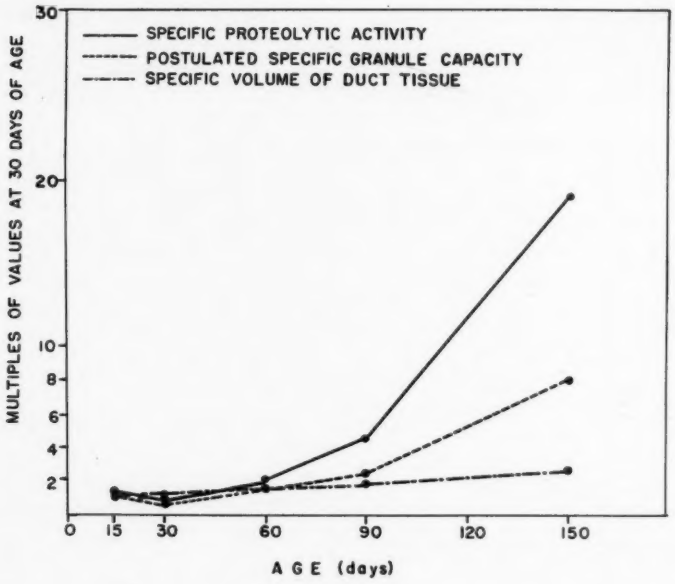

FIGURE 6

Specific proteolytic activity, granule capacity and duct mass with age (female rats).

tween 30 and 150 days of age, an eighteen-fold increase in male animals and an eight-fold increase in females would have to have occurred.

It was not found possible to estimate the number or size of granules of individual cells. Inspection showed that increasing numbers of the cells which make up the intralobular ducts became filled with granules. Because of the mixture of cells with different granule content, it is difficult to judge whether the observed increase in number and size alone allowed the increase in secretory capacity, or whether in addition the enzyme content of each granule increased.

3. *Sex Differences in the Submaxillary Glands of Rats*

At no age is it possible to tell the glands of males from those of females by microscopic inspection, as can readily be done in mice of like ages, nor do quantitative studies bring out any quantitative differences. In this respect, the present investigation confirms the findings of other workers (Lacassagne, '40).

Proteolytic activity, on the other hand, is clearly different in the two sexes from 60 days on. The difference grows to a maximum between 60 and 90 days. At 90 days, it is five times as high in males as in females. At 150 days, the difference in rats is of the same order as that found by Junqueira in mice, with males showing twice as much activity as females.

The comparison of males and females furnishes a clear cut instance of a biochemical difference which is not morphologically detectable. The number of granules never appeared to be larger in the male animals, even at the time when the proteolytic activity was five times as great as that of the females. If the cells in males had contained five times as many granules as those in the females, the granules would surely have appeared more tightly packed or the cells more bulging.

It seems, therefore, more likely that the enzyme content of the granules in the male was higher than that of the granules in the female. For this reason, castration should be expected to lead not only to a visible reduction in the number of granules, but also to a reduction of the secretory capacity of the granules themselves.

4. *Age Curve of the Postnatal Changes in the Submaxillary Gland*

The overall growth of the submaxillary gland follows much the same age pattern as the pancreas. (Figures by conversion from Donaldson's data; figure 7). Both organs show slower rates of growth between 60 and 90 days and resumption of the prepubertal rates in the weeks following. The slowed growth during puberty is indeed typical for total body growth.

The growth of the duct portion of the gland is slowed also, though much less than that of the entire gland. The proteolytic activity on the other hand shows an accelerated increase between 60 and 90 days of age. The only examples of similar timing of growth are organs like the prostate gland, that is, secondary sex organs which depend for their development on the male sex hormone. Figure 8 shows

that the main growth of the testes occurs between 30 and 60 days; the main growth of the prostate occurs between 60 and 90 days. In the male rats, the growth curve of proteolytic activity and with it the secretory capacity of the granules follow the same pattern as the prostate. It therefore appears probable that the increase in proteo-

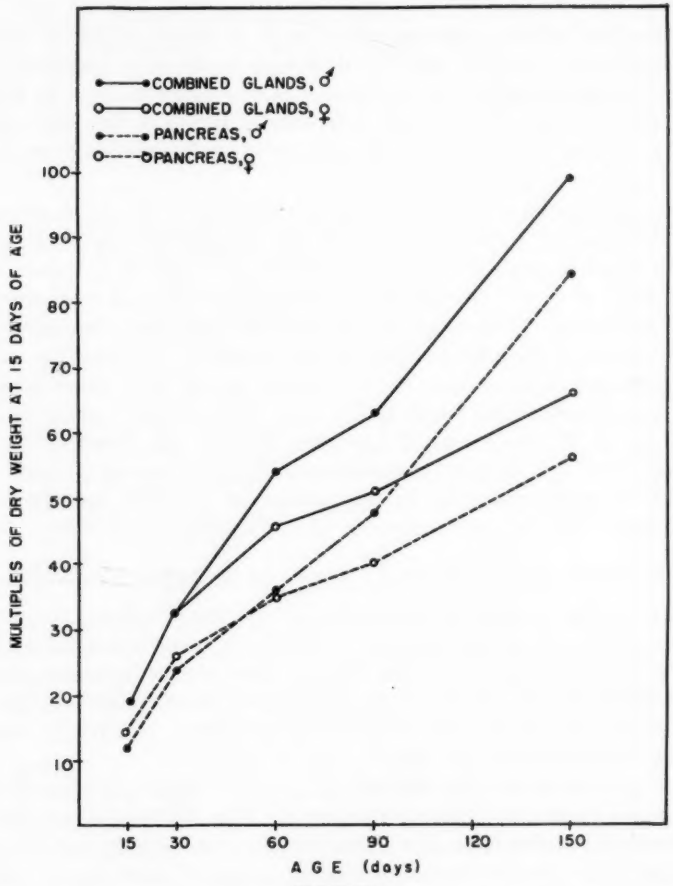

FIGURE 7

Changes in the dry weights of the combined glands and the pancreas in rats of different ages.

lytic activity during puberty occurs under the influence of the male sex hormone.

In the females, the 60 to 90 days period is a phase of more nearly steady increase than of accelerated increase in proteolytic activity. This finding, while not proving the hypothesis that the male sex hor-

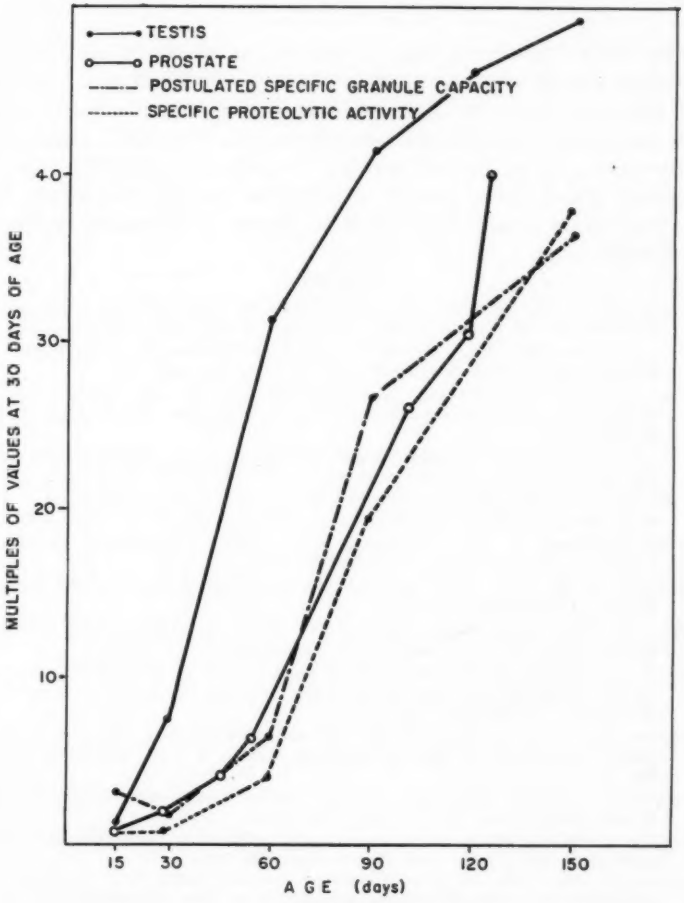

FIGURE 8

Growth curves of organs and functions in rats.

mone accounts for development of proteolytic activity in the males, fits in well with such a hypothesis.

The increase in the volume percentage of intralobular tissue, which if anything slows rather than accelerates between 60 and 90 days and which is alike in males and females, does not seem to be controlled by the sex hormone.

* * * * *

The study has shown that in the rat, as in the mouse, the submaxillary glands are the site of secondary sex differences. Although this difference finds no discernible structural expression in the rat, male and female animals are nevertheless found to differ functionally in the same manner in both species. In male rats and mice, the submaxillary gland has a greater capacity for protein breakdown, both by virtue of its greater size and of its higher specific activity, than in the females.

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INORGANIC CHEMICAL HETEROGONY IN MAN AND ANIMALS

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(Received for publication April 27, 1955)

"It is safe to say that when all exceptional cases have been considered and when all criticisms of the heterauxetic treatment have been taken into account, it nevertheless remains the most convenient way of comparing the chemical development of one organism with that of another."—Needham.

The development of the isotopic dilution method has made possible for the first time the measurement of certain aspects of total body composition in living man. In the course of some studies on the estimation of total body sodium in subjects of varying age by this method (Forbes and Perley '51), it became evident that the data could best be organized by use of the differential growth equation, $y = bx^k$, where x is total body sodium and y is body weight. Using data available in the literature, total body water was also fitted to a differential growth equation; total body potassium in the fetus could be treated similarly, and the calculated line extrapolated to include data on adults for this constituent (Forbes '52a). More recently, total body chloride has been found to conform to a differential growth equation over most of the growth span (Cheek '54, Forbes and Reid).

Differential growth constants have been calculated for Ca, P, N, K, Na, Cl, Fe and H_2O in the human fetus (Iob and Swanson '38, Hamilton '36), for all of these constituents in the young rat, and for water in the rat fetus (Iob and Swanson '38, Hamilton and Dewar '38), the former authors concluding that correspondence between the two species was rather good. Needham (1942) has presented data for total body ash in a number of vertebrate and non-vertebrate species.

Recently, information on changes in certain aspects of total body

This work was supported by a grant from the Playtex Park Research Institute. The assistance of Anne Lewis is gratefully acknowledged.

composition during growth has been presented for a number of mammalian species (Spray and Widdowson '51). Through the kindness of Dr. Widdowson the original data have been made available to me for calculation of the differential growth constants.

Presented herein are comparisons between man and the rat, cat, rabbit and pig with regard to total body content of water, sodium, and potassium over the growth span of each species. Data for total body chloride in man and the rat are also presented. Regression constants were calculated in each instance from the original data, using the method of least squares. Total body weight was chosen as the independent variable since this was the only parameter of body mass available for all.

With but few exceptions, the data can be quite satisfactorily represented by straight lines on a double logarithmic grid. In the case of total body potassium in the female rat, a change in slope occurs at age 50-70 days. The line for water in rat fetus differs somewhat from that found in postnatal rats, and two lines are needed to describe total body water in man—one for early fetal life and another for the period from mid-fetal life to post-natal maturity. There is a sharp break in the slopes of the regression lines for sodium and potassium in the human fetus at a weight of approximately 150 grams.

The source data are indicated in Tables 1-4 together with the slope k, the y axis intercept b, the standard deviation from regression, and the correlation coefficient for each regression equation. The calculations were made according to standard methods (Snedecor '46).

Sodium (Table 1 and Figure 1). Total body sodium in meq. is plotted against body weight in grams. The data indicate a close similarity in the rate at which sodium content changes during growth for the five species; in other words the decline in sodium content per unit weight proceeds in much the same fashion in each during growth. Indeed, the lines were grouped so closely that some of them had to be omitted from Figure 1 for the sake of clarity. The values of the standard deviations and correlation coefficients serve to indicate the goodness of fit in each instance.

As pointed out previously (Forbes and Perley '51), sodium "growth" in man shows a remarkable degree of consistency over most of the growth span, namely from the 260 gm. fetus to the 100 Kg. adult (plots of the individual points are illustrated in this article). There appears, however, to be a rather sharp break in the regression

slope in early fetal life with indications of a rapid rise in sodium content ($k > 1.0$) during the first months, and then the tendency for sodium content to fall begins abruptly and persists throughout growth. The great paucity of data for this stage of fetal life, and the

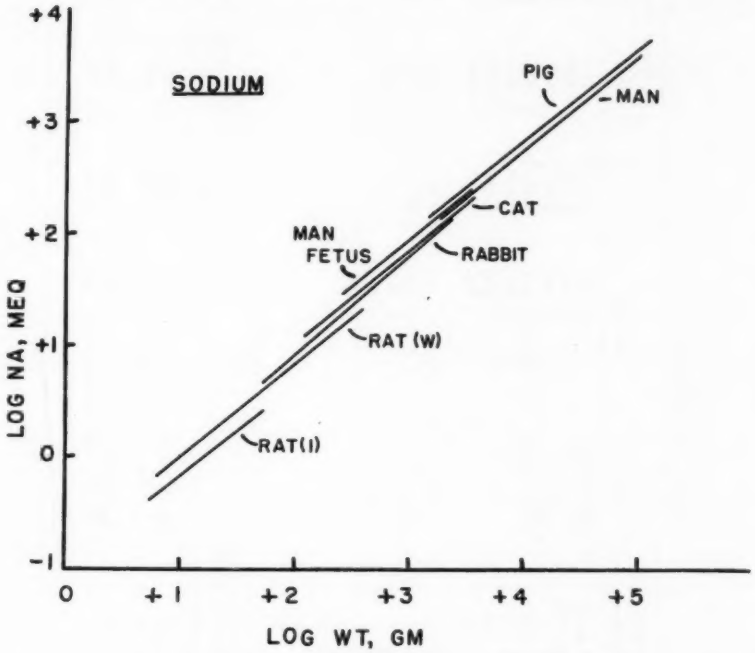

FIGURE 1

Log-log plots of body weight in grams versus total sodium content in milliequivalents for the five species. Regression constants are listed in Table 1. The line labelled "Rat (W)" refers to Spray and Widdowson's data on the male rat; "Rat (I)" is a plot of Iob and Swanson's data. Only the line for male rabbits is shown. (*) In many of the recorded series of analyses, the animals were grouped according to weight; the value in parentheses is the number of such groups available for purposes of calculation.

complete lack of data on animal fetuses with which to make comparison, makes any interpretation of this phenomenon rather hazardous.

Chloride (Table 2 and Figure 2). Data covering the growth span were available only for the rat and man. Our results using radiobromide dilution (Forbes *et al.* '53, Forbes and Reid) show a good

TABLE 1—SODIUM

Author	Source Data				Calculated Data			
	Species, sex	Number*	Age	Weight	k	b	s.d.	r
(29)	Rat, M	99 (11)	Birth-adult	6- 400 gm.	0.835	—.845	.030	.998
"	Rat, F	105 (11)	" "	6- 250 gm.	0.780	—.758	.054	.992
(19)	Rat	15	Birth-24 d.	5- 51 gm.	0.812	—.979	.030	.998
(29)	Rabbit, M	22 (7)	Birth-adult	50-2600 gm.	0.883	—.832	.052	.998
"	Rabbit, F	21 (7)	" "	50-2600 gm.	0.874	—.812	.040	.998
"	Cat, M&F	21 (6)	" "	120-3600 gm.	0.847	—.707	.023	.999
"	Pig, M&F	37 (6)	" "	1.5- 120 Kg.	0.823	—.446	.019	.9996
(3, 6, 16, 20, 21, 22)	Man	29	Fetus	260-3400 gm.	0.832	—.537	.047	.988
(9)	Man, M	46	Birth-34 y.	1.8- 100 Kg.	0.829	—.552	.057	.992
"	Man, F	9	10-27 y.	24- 82 Kg.	0.754	—.234	.028	.972
(20)	Man	4	Fetus	18- 115 gm.	1.51	—2.04		

TABLE 2—CHLORIDE

Author	Source Data				Calculated Data			
	Species, sex	Number	Age	Weight	k	b	s.d.	r
(19)	Rat	20	Birth-24 d.	6- 62 gm.	0.843	—1.141	.024	.997
(6, 17, 20, 22, 23, 25)	Man	33	Fetus	110-3300 gm.	0.808	— .624	.047	.986
(12, 13)	Man, M&F	29	Birth-33 y.	2.1- 88.7 Kg.	0.858	— .825	.052	.996
(4)	Man, M&F	50	Birth-16 y.	2.3- 52.7 Kg.	0.833	— .759	.034	.991

correspondence with those of Cheek ('54) who used stable bromide. It is also of interest that the calculated ratio of total sodium to total chloride content in man is approximately 1.4/1 at a body weight of 2000 gm. and 1.5/1 at 100 Kg., values which are fairly close to those

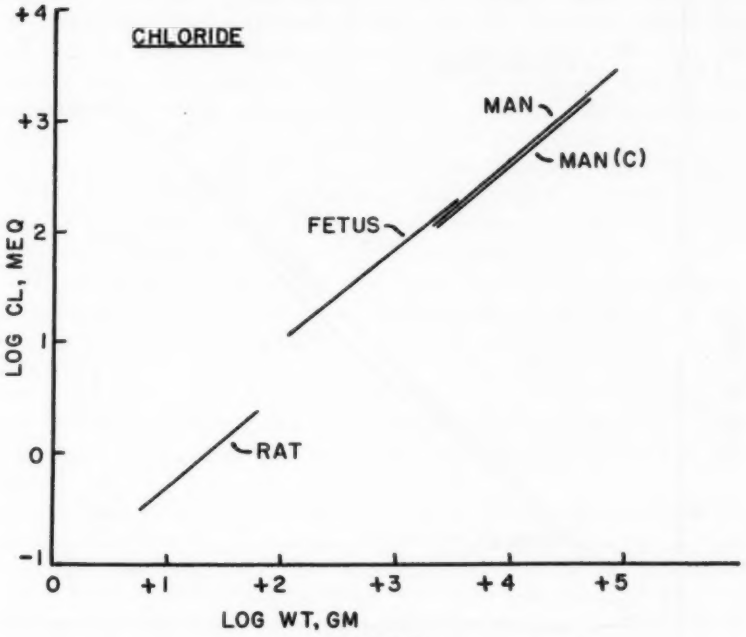

FIGURE 2

Log-log plots of body weight in grams versus total chloride in milliequivalents for rat and man. "Man (C)" is a plot of Cheek's data. Regression constants are listed in Table 2.

for the concentration ratio of these two substances in blood serum. A plot of post-natal data has been published by Cheek ('54).

It should be mentioned that there are two analyses of human fetuses recorded by Iob and Swanson ('34) which are not included in the equation listed in Table 2. These subjects weighed 24 and 59 grams, respectively, and contained much less chloride per kilogram body weight than the older fetuses. It would seem, therefore, that k is greater than one for this early period of fetal life, a situation

noted above for sodium. It did not seem appropriate, however, to calculate regression constants from such scanty data.

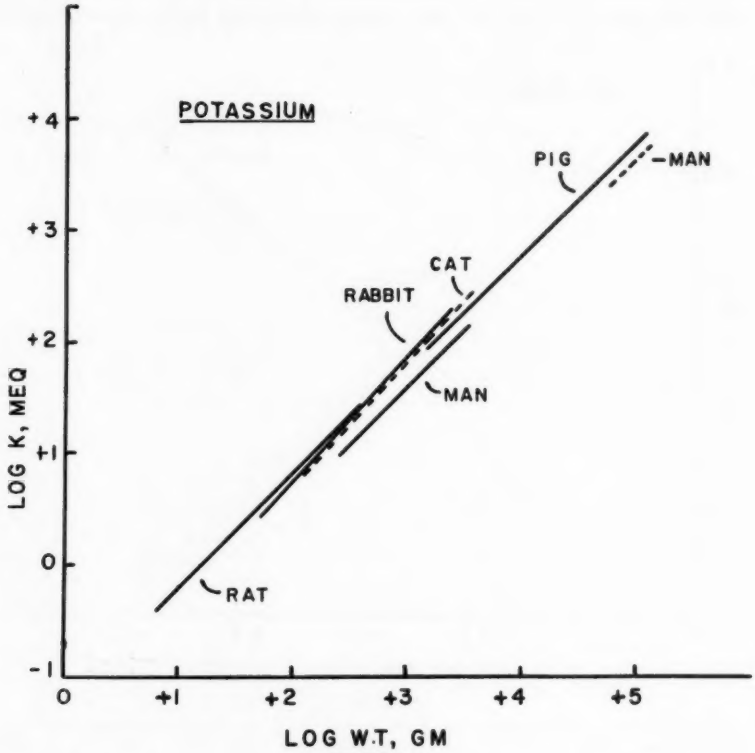

FIGURE 3

Log-log plots of body weight in grams versus total potassium in milliequivalents for the five species. Regression constants are listed in Table 3. The line labelled "rat" depicts Spray and Widdowson's data for the male rat; for the rabbit only the male data are shown. The dotted line for man is a direct extrapolation of the solid line based on fetal data, and fits the adult values (Corsa et al.) quite well. (*) is the number of animal groups.

Potassium (Table 3 and Figure 3). As in the case of sodium, the behavior of the various species with regard to potassium "growth" is fairly consistent. The close grouping of the various curves made it impossible to include all of them in Figure 3.

As mentioned previously, no data exist for total body potassium content in human infants and children, although measurements have been recorded for young adults. Regression constants were calculated from fetal data only, and the dotted portion of the line for man in Figure 3 represents an extrapolation to the adult weight range. It is of interest that this extrapolation does describe the adult values; the question of linearity during post-natal growth cannot be answered until data on this phase of the human growth curve are forthcoming.

Here, too, there is a break in the regression slope during early fetal life, similar in character to that noted above for sodium. A complete plot of the human data has been published elsewhere (Forbes '52a).

Two lines are needed to describe potassium "growth" in the female rat. It is probable that the pronounced decrease in slope at a body weight of 124-168 gm. is due in part to increase in body fat.

Water (Table 4 and Figure 4). Values for this constituent show an even better interspecies correspondence than those for Na, K, and Cl. In general, standard deviations are smaller and correlation coefficients larger, indicating a closer fit of the data to the individual regression lines. The close interspecies correspondence becomes more significant upon realization that the weight range represented is of the order of a million-fold. Not all of the lines could be included in Figure 4.

The change in regression slope during early fetal life in man occurs somewhat later than those noted above for Na and K; moreover, the data are sufficiently numerous to permit definition of the magnitude and location of the change in slope for water and calculation of the standard deviation and correlation coefficient for the early fetal period. A complete plot of the human data has been published elsewhere. (Forbes '52a).

Discussion

The data presented offer considerable support to Needham's concept of "the fundamental chemical ground-plan of animal growth" (1942). The fact that these particular aspects of chemical growth in man bear such a close relationship to those of other mammals is of considerable interest.

The linear relationship between the logarithm of total body sodium, chloride, potassium and water on the one hand, and the logarithm of body weight on the other, appears to hold true over a significant por-

TABLE 3—POTASSIUM

Author	Source Data: Species, sex	Number*	Age	Weight	Calculated Data: k	b	s.d.	r
(19)	Rat	15	Birth-24 d.	5- 51 gm.	1.074	—1.305	.018	.998
(29)	Rat, M	99 (11)	Birth-adult	6- 400 gm.	1.032	—1.234	.050	.995
"	Rat, F	60 (4)	Birth-50 d.	6- 124 gm.	1.142	—1.365	.022	.9996
"	Rat, F	45 (7)	70-321 d.	168- 253 gm.	0.439	+0.140	.014	.898
"	Rabbit, M	22 (7)	Birth-adult	50-2600 gm.	1.100	—1.441	.024	.9995
"	Rabbit, F	21 (7)	" "	50-2600 gm.	1.085	—1.397	.040	.999
"	Cat, M&F	21 (6)	" "	120-3600 gm.	1.111	—1.498	.024	.9994
"	Pig, M&F	37 (6)	" "	1.5- 120 Kg.	1.009	—1.255	.046	.998
(3, 6, 17, 20, 21, 22)	Man	28	Fetus	260-3400 gm.	1.022	—1.457	.055	.987
(5)	Man, M	29	21-32 y.	57- 125 Kg.				
(20)	Man	4	Fetus	18- 115 gm.	1.96	—3.33		

TABLE 4—WATER

Author	Source Data: Species, sex	Number*	Age	Weight	Calculated Data: k	b	s.d.	r
(16)	Rat	70 (8)	Fetus	.07- 6 gm.	.984	—.048	.004	1.0
(19)	Rat	43	Birth-24 d.	5- 62 gm.	.882	—.020	.021	.997
(16)	Rat	79 (10)	Birth-adult	6- 260 gm.	.950	—.040	.012	.9998
(29)	Rat, M	99 (11)	" "	6- 400 gm.	.914	+.016	.019	.9994
(29)	Rat, F	105 (11)	" "	6- 250 gm.	.886	+.044	.028	.998
(7)	Rabbit	22	Fetus-14 d.	0.6- 200 gm.	.960	—.029	.018	.9995
(29)	Rabbit, M	27 (7)	Birth-adult	50-2600 gm.	.971	—.047	.028	.999
(29)	Rabbit, F	21 (7)	" "	50-2600 gm.	.951	+.004	.021	.999
(29)	Cat, M&F	21 (6)	" "	120-3600 gm.	.935	+.041	.010	.9999
(29)	Pig, M&F	37 (6)	" "	1.5- 120 Kg.	.937	+.115	.025	.9994
(7, 20, 27)	Man	36	Fetus	1- 370 gm.	.988	—.022	.021	.9995
"	Man	21	Fetus	490-3300 gm.				
(8, 14)	Man, M&F	27	Birth-12 y.	2.3- 60 Kg.	.913	+.167	.042	.998
(26)	Man, M&F	29	18-32 y.	46- 85 Kg.				

tion of the growth span of each of the five species under study. Indeed, in most instances the change in body weight represented is of the order of 50 to 100-fold; in one (body water in man) it is 170-fold.

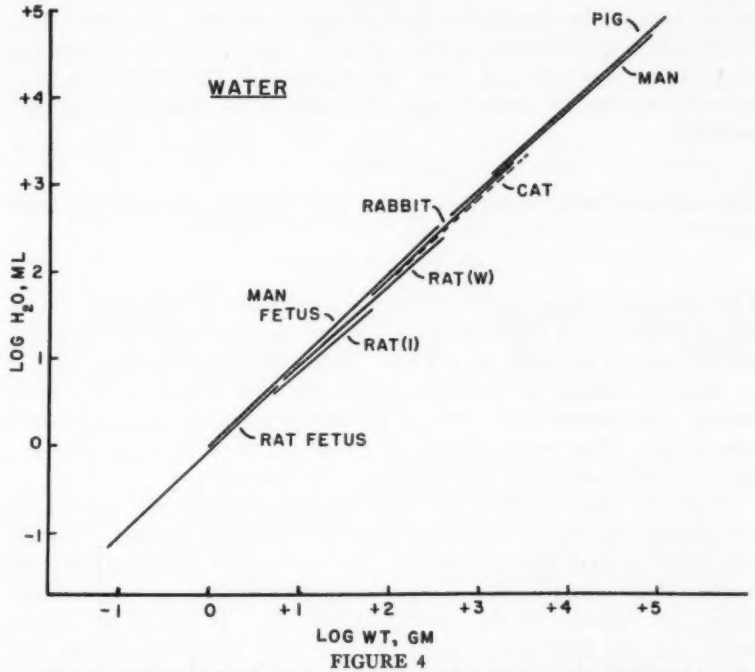

FIGURE 4

Log-log plots of body weight in grams versus total body water in millimeters for the five species. The designations "W" and "I" refer to male rats of Spray and Widdowson and the series of Iob and Swanson respectively. Only the line for male rabbits is shown; that for the cat is dotted for purposes of clarity. Regression constants are listed in Table 4. (*) is the number of groups.

However, it must be pointed out that it has not been possible to encompass the *entire* growth span with a single equation in the few instances for which data are available. There is a definite change in the regression slope for water at mid-fetal life in man, and at birth in the rat. Similar changes in early fetal life were noted for Na, K, and Cl but are less well documented and more data are needed.

The significance of the differential growth equation, $y = bx^k$, has been discussed at length by others (Needham '42, Huxley '32,

Adolph '49, Brody '45). There seems to be little doubt that equations of this general type can be used to describe a variety of body attributes—physical, physiological, and chemical—during the growth of a single species and to make interspecies comparisons. Brody has suggested that x^k be designated "physiological weight", in contrast to $x^{1.0}$ which is gravitational weight, since it bears a linear relationship to y. From one point of view, the equation is the expression of a ratio of two specific growth rates, as follows: the differential growth equation

$$y = bx^k \qquad (1)$$

differentiated with respect to time, and divided through by itself gives

$$dy/ydt = k.dx/xdt \qquad (2)$$

Thus the percentage increment of the constituent in question, for example sodium, is a constant proportion of the percentage, or specific, growth rate of the body as a whole. When the latter is rapid, as it is in infancy, change in sodium content per unit weight occurs more rapidly with growth than later in childhood when the specific growth rate is less. This type of biphasic curve has actually been observed for Na, H_2O, and Cl in man (Forbes '52b, Cheek '54).

From the data at hand it would appear that, for the most part, mammalian growth is associated with a decline in sodium, chloride, and water content per unit weight, and an increase in potassium; and further that within certain limits the rate of change in body composition is a function of the specific growth rate. It should be remembered that the information at hand refers only to the total body content of the particular constituents and does not allow for partition of the body into intra- and extra-cellular compartments. A study of other chemical constituents, such as Ca, P, N, etc., was not considered feasible at this time in view of the paucity of data on post-natal man.

The question naturally arises as to the validity of the isotopic dilution method for the measurement of total body composition in man. The deuterium oxide method would appear to give a close approximation to total body water in both infant and adult (Friis-Hansen et al. '51, Schloerb et al. '50). The matter of Na^{24} dilution has been discussed previously (Forbes and Perley '51) and it would appear that this method results in values for newly born infants which are

very close to those obtained by carcass analysis. Because of the slow rate of exchange of Na^{24} in adult bone, however, it is probable that this method gives results which are somewhat low in the adult. Bromide dilution would appear to yield accurate results in newly born infants (Forbes et al. '53), but there are no direct measurements of total body chloride in adult humans available for comparison in this age group. No measurements of total body potassium have been made in infants and children by the K^{42} dilution method. However, from consideration of various tissue analyses Corsa et al. ('50) concluded that 95% of the total body content was detectable by this method in the adult. It is of interest that the scatter in the data obtained by isotopic dilution is roughly the same as that obtained by carcass analysis in man.

Whatever the theoretical considerations of the differential growth equation may be, its use has one practical consequence—namely the prediction of mineral and water retentions the result of normal growth. Differentiation of equation (1) with respect to x gives

$$dy/dx = k \cdot y/x \qquad (3)$$

so that the retentions of Na, K, Cl and H_2O occurring as a result of normal growth can be closely approximated if the initial weight and weight gain of the subject under study are known. Since the above equation is a differential one, the approximation resulting from such a calculation will be valid only if the observational period is short. Taking as an example a 4000 gm. child who gained 20 gm. (as a result of normal growth) on a given day: Na 1.2 meq., Cl 0.70 meq., K 0.9 meq., and H_2O 13.0 ml. would be incorporated in the growing tissues of this subject on this particular day. Perhaps information of this sort could be of assistance in the interpretation of metabolic balance studies.

Conclusions

A close similarity between five mammalian species, including man, with regard to certain aspects of chemical growth has been demonstrated by means of the differential growth equation, $y = bx^k$. With but few exceptions the total body content of Na, Cl, K, and H_2O for each species follows a straight line when plotted against body weight on a double logarithmic grid.

In general, the regression slopes are grouped in the range of 0.75

to 0.88 for sodium, 0.81 to 0.86 for chloride, 1.01 to 1.14 for potassium, and 0.88 to 0.99 for water. The occasional exception to this general trend is noted.

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A LONGITUDINAL STUDY OF CHANGE IN SIZE AND FORM OF THE LOWER LIMBS ON NORTH AMERICAN WHITE SCHOOLBOYS

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(Received for publication June 9, 1955)

This report pertains to growth of the lower extremities in 70 nonpathologic white boys examined annually during the postnatal period from age 5 years to age 11 years.[1] Study is made for three morphologic variables, two of size and one of form. Lower limb length and leg girth are the size variables; the form variable is leg girth in percentage of lower limb length. The scope of data treatment is presaged by the captions under which findings are colligated: central tendency, variability, correlation, and individual growth pattern.

Viewed broadly, the present investigation constitutes the laying of another piece in a mosaic of research on body size and form during childhood. That is, besides having its intrinsic design, the investigation contributes to larger designs on age changes in limb size (7, 14, 16, 21), secular trends in child morphology (11, 12, 13, 27), age changes in body form (4, 9, 26, 30), associations among measures of morphologic status and velocity (19, 23, 25, 31), intra- and interindividual growth trends (1, 10, 20, 22) and normative reference frames for limb size and form (17, 24, 28, 29).

MATERIALS

Source. The original materials were obtained on 70 white schoolboys residing in or near Iowa City, Iowa. Thirty-seven of the boys were enrolled for study during the years 1940-42 and 33 during the years 1946-48. The former attended the State University of Iowa experimental elementary school and were examined during school hours; the latter participated in a separate long-term research program and were examined by special appointment.

All of the subjects were American-born white boys. Information

[1] The term "growth" is used with the connotation recognized in the founding of this journal. It encompasses changes during the life history of an organism in the size, form, number, kind, location, and texture of its somatic constituents (15).

on ethnic background showed that for 56, or 80 per cent, the four grandparents were immigrants or descendants of immigrants from northwest Europe (the British Isles, Germany, Scandinavia, France, or the Netherlands). An additional 17 per cent had (a) three grandparents of northwest Europe ancestry and one of central European ancestry or (b) two grandparents of northwest European lineage and two of eastern or central European lineage. The small percentage remaining comprised two boys whose progenitors were immigrants from eastern Europe.

With reference to socioeconomic status, the subjects were predominantly from the professional and business proprietor groups. Analysis of data on occupation of the fathers gave the following: in the professional and managerial categories, 83 per cent; in the commercial and skilled trades categories, 11 per cent; and in the semiskilled and unskilled categories, 6 per cent. Upward socioeconomic selection also is reflected in the fact that the boys were drawn partly from a university elementary school (attendance involving application for enrollment, moderate tuition, and transportation expense) and partly from a long-term research program (participation entailing likelihood of continuing residence in the community, interest in supplying developmental data, and willingness to keep seriatim appointments over a period of years).

Method. Detailed description of the anthropometric techniques used in obtaining the original data has been presented in an earlier paper (19). Other publications have reported on the reliability of these procedures (6, 8). Consequently, it is sufficient here to identify the measures succinctly and make brief reference to their precision.

The three measures of size and form are:

1. Lower limb length. Stature minus stem length, i.e., the maximum vertical distance from the most inferior plane of the ischia to the most inferior plane (plantar surface) of the feet.

2. Leg girth. Maximum circumference of the left leg in the region of the calf.

3. Lower limb index. The ratio of leg girth to lower limb length multiplied by 100.

Values for each measure were derived through exceptionally rigorous anthropometric methods. At every examination the basic measurements of leg girth, stature, and stem length were made by two anthropometrists. In instances where their results failed to show

close agreement (differed by more than 0.1 cm. for leg girth, 0.2 cm. for stature, or 0.4 cm. for stem length), additional measurements were taken. It follows that each record utilized was the average of two or more separate measurements. These high laboratory standards were developed and maintained in order to secure values adequate for investigating individual patterns of morphologic growth.

Each subject was measured annually over the period extending from age 5 to age 11 years. Always the examinations were made within a few days of a subject's birthdate. In other words, the materials are fully longitudinal and regular—they comprise values for all three measures at 7 consecutive birth anniversary intervals.

Central Tendency

Descriptive. Table 1 presents arithmetic means at successive annual ages for the three traits studied. The curves obtained by plotting each series of means on age are portrayed in Figure 1. These tabular and graphic materials, representing contemporary North American white boys of northwest European descent and above-average socioeconomic level, support the following generalizations:

1. Over the childhood age period from 5 years to 11 years, average length of the lower extremities follows a rising trend concave to the base line. That is, the average growth of boys in lower limb length between ages 5 and 11 years is a negatively accelerated increasing function of age. Mean increases for the biennial segments 5 to 7 years, 7 to 9 years, and 9 to 11 years are 8.0 cm., 7.1 cm., and 6.3 cm., respectively. Annual percentage increments approximate 8.3 between 5 and 6 years, 6.5 between 7 and 8 years, and 4.6 between 10 and 11 years.

TABLE 1

Arithmetic Means for Size and Form of the Lower Limb. (Based on seriatim records amassed 1940-54 from annual measurement of 70 Iowa City schoolboys mainly of northwest European descent and above-average socioeconomic status.)

Age (yrs.)	Lower Limb Length (cm.)	Leg Girth (cm.)	Lower Limb Index (%)
5	48.5	23.1	47.7
6	52.5	24.2	46.0
7	56.5	25.3	44.8
8	60.2	26.5	44.1
9	63.6	27.7	43.6
10	66.8	28.9	43.4
11	69.9	30.2	43.2

2. The average absolute magnitude curve for leg circumference from ages 5 to 11 years is a rising trend closely approaching rectilinearity, but somewhat convex to the base line. In other words, average growth of boys in leg girth between 5 and 11 years departs slight-

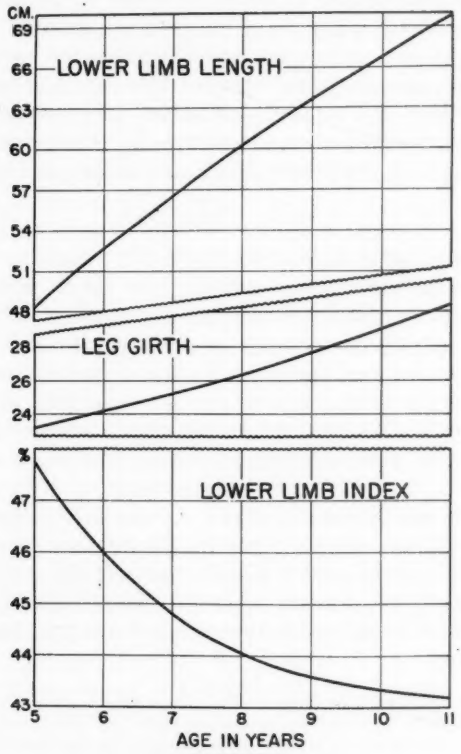

FIGURE 1
Curves drawn to the means presented in Table 1.

ly from an increasing linear function of age in the direction of positive acceleration. The mean increases for consecutive biennial periods are 2.2 cm., 2.4 cm., and 2.5 cm. Relative increments approximate 4.5 per cent annually throughout the 6-year period, i.e., the slightly increasing annual centimeter gains are just sufficient to maintain constant annual percentage increments.

3. During the age interval from 5 years to 11 years, the average course for the ratio of leg circumference to length of the lower extremities is a falling curve convex to the base line. That is, the average male index trend for leg girth in percentage of lower limb length between ages 5 and 11 years is a negatively accelerated decreasing function of age. Decreases in the mean index over successive biennial periods are 2.9, 1.2, and 0.4. Annual relative declines approximate 3.6 per cent between 5 and 6 years, 1.6 per cent between 7 and 8 years, and 0.3 per cent between 10 and 11 years.

Comparative. Previous investigations have compared findings for average size of the lower extremity on North American boys representing different ethnic (7) and socioeconomic (18) groups. The comparative section of this paper is focused upon the problem of secular change.

The early research literature on child growth was examined to locate studies similar to the present study in method of data collection and sample selection, except that drawing of the samples occurred prior to 1900. Due to the restricted scope of investigations reported during the nineteenth century, it was not possible to obtain averages for lower limb length and leg girth from the same report. Of the materials available for comparative use the most appropriate are supplied in studies by MacDonald (5) and Hall (3).

MacDonald affords means for lower limb length on white boys 7 to 11 years of age drawn ca. 1897 from the school population of Washington, D. C. The boys selected "were of American parentage" (both parents born in North America) and "belonged to the nonlaboring classes" (fathers in managerial or professional occupations). Means at 7, 9, and 11 years of age (N's = 197, 311, and 343) are 53.0 cm., 59.0 cm., and 64.6 cm. Reference to Table 1 shows that the corresponding means of the present study are higher by 3.5 cm., 4.6 cm., and 5.3 cm.[2]

Hall analyzed data for leg circumference collected 1891-93 on a sample of 127 boys 11 years of age attending private schools in or near Philadelphia. Their parents were "almost without exception born in America, and the families came originally from England" (3, p. 21). With "very few exceptions" the families were "well-to-do"; and the schools offered "exceptional opportunities" for physical de-

[2] From the standpoint of statistical dependability, differences as small as 1.5 cm. would be significant at the 1 per cent level of confidence.

velopment. The measurement taken was "girth of the calf . . . at its greatest diameter" (3, p. 24). Hall reports a median of 26.5 cm., which is 3.4 cm. less than the median at 11 years (29.9 cm.) for the boys of the present study.[3]

The foregoing comparisons lead to the following inference regarding 11-year-old North American white boys of northwest European descent and above-average socioeconomic level: in relation to the average boy of half a century ago, the average boy of today is larger by fully 8 per cent in lower limb length and by roughly 13 per cent in leg girth.

Variability

Absolute measures. Table 2 describes, at successive annual ages, the dispersion of the 70 records each for lower limb length, leg girth, and lower limb index. Selected findings from this table are:

1. Throughout the boyhood period covered there is an increase in the absolute variability of all three traits. The standard deviations for lower limb length rise from 2.3 cm. at 5 years to 3.6 cm. at 11 years, those for leg girth from 1.6 cm at 5 years to 3.0 cm. at 11 years, and those for lower limb index from 2.9 per cent at 5 years to 3.9 per cent at 11 years.

2. The distributions for lower limb length are roughly symmetrical and bell-shaped; those for leg girth and lower limb index are skewed to the right. In the case of lower limb length, the average centimeter distance between the 5th and 25th percentiles is the same as that between the 75th and 95th percentiles. The average distances between percentiles 75 and 95 exceed those between percentiles 5 and 25 in leg girth by 1.5 cm. and in lower limb index by 1.8 per cent.

3. There is less overlapping of the successive distributions for lower limb length than for leg girth. The 95th percentile of the lower limb length distribution at age 5 years approximates the 5th percentile two years later; the 95th percentile of the leg girth distribution at age 5 years approximates the 5th percentile six years later.

4. Normative utilization of the percentiles rests on an explicit percentage subdivision of each frequency surface: 5, 20, 50, 20, 5. Under this apportionment, lower limb lengths for boys 5 years of age may be termed short (below 44.7 cm.), moderately short (44.7 cm. to

[3] Again, a difference no larger than 1.5 cm. would be significant statistically at the 1 per cent confidence level.

46.6 cm.), average (46.7 cm. to 50.2 cm.), moderately long (50.3 cm. to 52.2 cm.), and long (above 52.2 cm.). Similarly, indices of lower limb form at age 5 years are categorizable as slender (below 43.7 per cent), moderately slender, average, moderately stocky, and stocky (above 53.2 per cent).

TABLE 2

Standard Deviations and Selected Percentiles for Size and Forms of the Lower Limb. (Based on longitudinal data accumulated 1940-54 from yearly examination of 70 Iowa City schoolboys mainly of northwest European ancestry and above-average socioeconomic status.)

Age (yrs.)	Standard Deviation	Percentiles: 5	25	75	95
		Lower limb length (cm.)			
5	2.32	44.7	46.6	50.3	52.2
6	2.57	48.4	50.4	54.5	56.6
7	2.79	52.1	54.3	58.7	61.0
8	3.01	55.5	57.9	62.5	65.0
9	3.19	58.5	61.3	65.9	68.6
10	3.43	61.3	64.5	69.2	72.2
11	3.61	63.9	67.5	72.3	75.6
		Leg girth (cm.)			
5	1.62	20.6	21.9	24.2	26.3
6	1.87	21.4	22.9	25.3	27.8
7	2.14	22.2	23.9	26.4	29.3
8	2.35	23.1	24.9	27.6	30.8
9	2.56	24.1	26.0	28.8	32.3
10	2.88	25.0	27.0	30.2	34.2
11	3.04	26.1	28.1	31.7	36.2
		Lower limb index (%)			
5	2.88	43.7	45.7	49.4	53.2
6	3.09	41.8	43.9	47.8	51.5
7	3.25	40.6	42.6	46.7	50.5
8	3.47	39.5	41.6	46.0	50.0
9	3.59	38.8	41.0	45.8	49.7
10	3.77	38.4	40.7	45.6	49.8
11	3.89	38.3	40.6	45.5	50.0

Annual differences. Each of the 70 subjects had records representing successive annual ages between 5 years and 11 years; from these records, measures of change in lower limb size and form were obtained for 6 consecutive one-year periods. Variability of the derived values, specific for trait and postnatal age interval, is displayed in Table 3. (It is not necessary to table the means for annual change since these are obtainable from the central tendency columns of Table 1, e.g., between 5 and 6 years of age mean increase in lower limb length is 4.0 cm., mean increase in leg girth 1.1 cm., and mean decrease in lower limb stockiness 1.7 per cent.) Table 3 shows:

1. Absolute variability of the three series of distributions for annual differences does not increase or decrease systematically over the age span studied. The standard deviations on leg girth are identical for the age intervals 5 to 6 years and 10 to 11 years. Those on lower limb length are alike for age intervals 7 to 8 years and 9 to 10 years, those on lower limb index alike for age intervals 6 to 7 years and 9 to 10 years.

TABLE 3

Standard Deviations and Selected Percentiles for Annual Change in Size and Form of the Lower Limb. (Derived from consecutive birthday-anniversary measurements on 70 Iowa City schoolboys mainly of northwest European ancestry and above-average socioeconomic status.)

Age Interval (years)	Standard Deviation	Percentiles: 5	25	75	95
		Lower limb length (cm.)			
5- 6	0.46	3.3	3.8	4.4	4.9
6- 7	0.39	3.2	3.7	4.2	4.7
7- 8	0.37	3.0	3.5	4.0	4.5
8- 9	0.36	2.8	3.2	3.7	4.2
9-10	0.37	2.6	3.0	3.5	4.0
10-11	0.40	2.3	2.8	3.3	3.8
		Leg girth (cm.)			
5- 6	0.41	0.4	0.7	1.2	1.8
6- 7	0.36	0.6	0.8	1.3	1.9
7- 8	0.34	0.7	0.9	1.4	2.0
8- 9	0.40	0.7	0.9	1.4	2.1
9-10	0.43	0.7	1.0	1.5	2.1
10-11	0.41	0.8	1.0	1.5	2.1
		Lower limb index (%)			
5- 6	0.72	—2.6	—2.2	—1.3	—0.4
6- 7	0.52	—2.0	—1.6	—0.8	—0.1
7- 8	0.48	—1.5	—1.0	—0.5	+0.1
8- 9	0.49	—1.2	—0.8	—0.2	+0.6
9-10	0.52	—1.0	—0.6	0.0	+0.8
10-11	0.48	—0.8	—0.5	+0.2	+0.8

2. The increment distributions for lower limb length approximate symmetrical surfaces; the corresponding distributions for leg girth and lower limb index are positively skewed. Compared with the average distances between percentiles 5 and 25, the average distances between percentiles 75 and 95 are the same for lower limb length, larger by 0.4 cm. for leg girth, and larger by 0.7 per cent for lower limb index.

With respect to use of the percentile frames of reference, an increment in lower limb length of 3.5 cm. transposes to a moderately

small gain for the 6th year, but a moderately large gain for the 11th year. Correspondingly, a decrement in lower limb index of —0.2 per cent transposes to an unusually small reduction in limb stockiness for the 6th year, yet symbolizes average expectancy for the 11th year.

CORRELATION

Lower limb length. There is a high positive relationship between lower limb length at age 5 years and lower limb length at age 11 years. Explicitly, the Pearson product-moment coefficient of correlation (r) is 0.91. The reduction in errors of prediction by reason of this coefficient (the index of forecasting efficiency, E) is 59 per cent. With regard to predicting (estimating) lower limb length for the older age, the standard error of estimate would be (a) 3.6 cm. by use of the mean at 11 years and (b) 1.5 cm. by use of lower limb length at 5 years in the best-fit rectilinear regression equation. It follows that the latter procedure affords an improvement of 59 per cent on the "best guess" standard error of 3.6 cm.

Variability in lower limb length at age 11 years can be viewed as a composite of variability at age 5 years plus variability in gain between 5 and 11 years. From this viewpoint, the above correlation for lower limb length at ages 5 and 11 years is a special case of part-whole correlation. The association between lower limb length at age 5 years and increase in lower limb length from 5 to 11 years is $r = 0.54$,[4] and the corresponding E = 16 per cent. This percentage advantage in the prediction of increments for lower limb length accrues by reduction of the standard error of estimate from 1.8 cm. when the mean increment is used, to 1.5 cm. when use is made of a best-fit rectilinear equation based on the 0.54 coefficient of correlation. It will be seen that the magnitude of the standard error of estimate is the same (1.5 cm.) whether one uses lower limb length at age 5 years to predict (a) lower limb length at age 11 years or (b) gain in lower limb length between 5 and 11 years.

Study was made of the association between increase in lower limb length during the age period 5 to 8 years and increase during the age period 8 to 11 years. Relational and predictive findings are: $r = 0.54$; the regression equation for predicting centimeter gain between 8 and

[4] This coefficient first was obtained by direct calculation, and then checked using the part-whole r and S.D.'s to derive the part-remainder r. For further particulars see reference 2, p. 357.

11 years = .51 (gain from 5 to 8 years) + 3.8; and the standard error of prediction by this equation = 0.9 cm. It follows the odds are 19 to 1 that a boy whose gain from 5 to 8 years is 11.0 cm., from 8 to 11 years will gain between 7.7 cm. and 11.1 cm. This represents a 16 per cent error reduction, since estimation in terms of the mean 8 to 11 increase gives 19 to 1 odds within the limits of 7.8 cm. and 11.8 cm.

Leg girth. The relationship between leg girth at age 5 and leg girth at age 11 is positive and moderately high ($r = 0.81$). There is a predictive efficiency conjoined with this correlation of 41 per cent. For example: In estimating leg girth on individual boys age 11, the standard error is reduced from 3.0 cm. using the age mean, to 1.8 cm. using the best-fit equation for rectilinear regression of leg girth at 11 years on leg girth at 5 years.

Investigation of correlation and prediction with respect to the two variables leg girth at age 5 years, and increase in leg girth between ages 5 and 11 years, yields 0.43 as the Pearson product-moment coefficient and 10 per cent as the accompanying index of forecasting efficiency. In absolute terms, where leg girths of boys 5 years of age are employed as the independent variable, predictions of gain in leg girth over the ensuing 6-year age span, in common with predictions of leg girth at age 11 years, have a standard error of 1.8 cm. Again, the two *r*'s are interpretable as instances of part-whole (0.81) and part-remainder (0.43) correlation.

The order of association between increases in leg girth during the first and last halves of the period under study approximates that reported in the previous section from parallel analysis of increment data for lower limb length. Explicitly, $r = 0.59$; E = 20 per cent; the best-fit rectilinear regression equation for predicting centimeter gain in leg girth between 8 and 11 years = .66 (gain from 5 to 8 years) + 1.59; and the standard error of estimate in application of this formula = 0.9 cm.

Lower Limb Index. The relationship between lower limb index at 5 years and lower limb index at 11 years is about as strong as that for leg girth at these ages. Specifically, $r = 0.78$ and E = 37 per cent. Similarity ceases at this point; there is no association between lower limb index at 5 years and change in lower limb index from 5 to 11 years ($r = 0.04$) and only low positive correlation between index

change from 5 to 8 years and index change from 8 to 11 years ($r = 0.33$, E = 6 per cent).

Earlier studies (24, 30) have found that in childhood relationships are not high between body form indices representing stockiness of the stem, upper extremity, and lower extremity. The present study contributes to the problem of determining the persistence of body form. It finds that while there is a tendency for boys 5 to 11 years of age to maintain their positions in peer distributions of lower limb index, this tendency falls far short of supporting the hypothesis that the ratio of leg girth to lower limb length is a permanent characteristic of the individual.

Other correlations. Three further relationships were investigated: (a) lower limb length with leg girth at age 5 years, (b) lower limb length with leg girth at age 11 years, and (c) increase in lower limb length between 5 and 11 years with increase in leg girth over the same 6-year period. Inspection of three scatter diagrams suggested slightly curvilinear associations. Consequently, both Pearson product-moment coefficients and correlation ratios were calculated. It was confirmed that r underestimates each relationship.

Eta coefficients are 0.63 for the regression of lower limb length on leg girth at age 5 years, 0.59 for the corresponding regression at age 11 years, and 0.59 for the regression of gain between 5 and 11 years in lower limb length on gain over the same period in leg girth.

Individual Growth Pattern

Lower limb length. Figure 2 graphically displays individual growth curves for lower limb length on 12 of the 70 boys studied. Discussion will proceed with reference to each panel in turn.

Shown in the first panel are 4 curves which represent a curvilinear course of growth over the age period from 5 to 11 years. For each of these boys, plus 7 others, increase in lower limb length between 5 and 8 years is greater than increase between 8 and 11 years by 3.0 cm. or more. Gain during the first half of the 6-year period surpasses gain during the last half of the period by 2.0 cm. to 2.9 cm. for 27 boys, and by 1.0 cm. to 1.9 cm. for 25 boys. Summing, there are 63 curves (90 per cent of those examined) exemplifying a growth type or pattern for boys in lower limb length that is a negatively accelerated increasing function of age.

The middle panel depicts individual growth curves for lower limb length that are approximately rectilinear. These 4 trends represent a group of 7. In other words, 10 per cent of the boys studied gained practically the same amount from year to year. For each of these in-

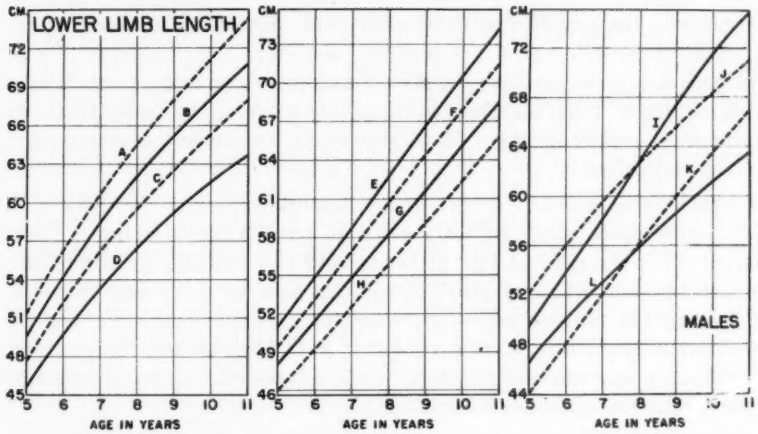

FIGURE 2

Twelve individual growth curves for length from inferior plane of ischia to plantar surface of foot.

dividuals, there is a difference of less than 1.0 cm. between the increase from 5 to 8 years of age and that from 8 to 11 years of age. Combining the findings of this and the preceding paragraph, individual patterns of male growth in lower limb length between ages 5 and 11 years may be characterized as rising curves varying in trend from rectilinearity to moderately strong curvilinearity concave to the base line.

The last panel of Figure 2 illustrates individual differences in lower limb length with respect to (a) increase during the period 5 to 11 years and (b) position in the group at successive ages. Boys I and L represent the increment extremes for the 6-year period, the former gaining 17.0 cm., or 36 per cent, and the latter 25.3 cm., or 51 per cent. The lower limb length of boy I exceeds that of boy L by 2.9 cm., or 6 per cent, at age 5 years and by 11.2 cm., or more than 17 per cent, at age 11 years. Boy J moves from a percentile rank of

93 in the lower limb length distribution at age 5 years to a percentile rank of 64 at age 11 years; change of position in the group for boy I is from 69 at 5 years to 91 at 11 years. Similarly, boys K and L shift from percentile ranks of 3 and 24 at 5 years to reversed ranks of 26 and 4 at 11 years.

Leg girth. Twelve of the 70 individual growth patterns for leg circumference are exhibited in Figure 3. The objective in selection

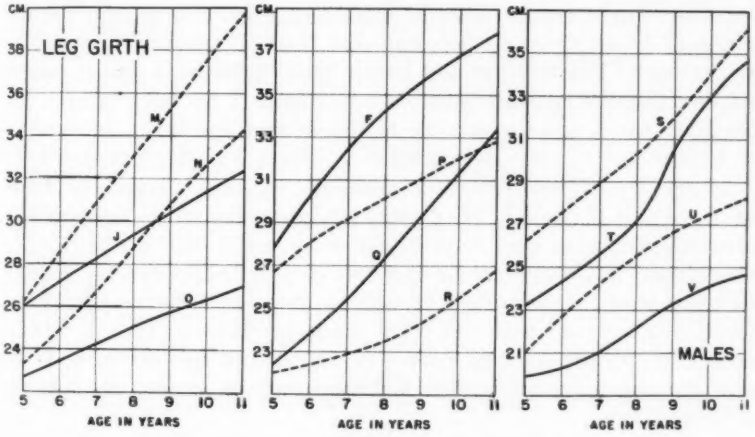

FIGURE 3

Twelve individual growth curves for maximum circumference of calf.

was the same as for Figure 2, i.e., to illustrate variations in trend slope and level among boys 5 to 11 years of age.

Thirty-one leg girth curves approximate rectilinearity. In none of the 31 does the gain between 5 and 8 years differ from that between 8 and 11 years by more than 0.5 cm. The first panel of Figure 3 shows 4 representatives of this series. Eighteen curves are moderately convex to the base line (the gain from 5 to 8 years is 0.6 cm. to 1.5 cm. less than that from 8 to 11 years) and 8 more markedly convex. Illustrative of the latter are the Figure 3 trends for boys R and S. Six curves are moderately concave to the base line (the 5 to 8 gain is 0.6 to 1.5 cm. more than the 8 to 11 gain) and 3 more strongly concave (trends F and U of Figure 3 are examples). Of the 4 curves remaining, 3 follow a moderately convexo-concave course (one of

these is trend V of Figure 3) and the other shows a linear course between 5 and 7 years succeeded by a bold convexo-concave rise (see trend T of Figure 3). Generalizing, 94 per cent of the leg girth curves are simple rising trends varying from positive acceleration (37 per cent) through constant acceleration (44 per cent) to negative acceleration (13 per cent); the additional 6 per cent are rising curves of greater complexity.

The range of increment in leg circumference between 5 and 11 years is represented by boys M and O of Figure 3. M has an increase of 13.6 cm., or 52 per cent, and O an increase of 4.3 cm., or 19 per cent. The smallest and largest gains between 5 and 8 years, 1.5 cm. and 6.8 cm., are portrayed in Figure 3 by boys R and M. Similarly for 8 to 11 years, the minimum and maximum increments of 1.9 cm. and 7.4 cm. are exhibited in Figure 3 by boys O and T.

At age 5 years, the percentile ranks for leg circumference on boys M, J, N and O are 93, 91, 61 and 47, respectively. Corresponding percentile ranks at age 11 years differ by +5, —9, +26 and —36. Boys P and Q have percentile ranks at 11 years of 83 and 84. Ranks on these boys at 5 years are 96 and 38, differing from the standings for age 11 by —13 and +46. The group positions of boys F and V remain practically constant from 5 to 11 years of age, the latter at or near the lowest percentile rank and the former at or near the highest percentile rank.

Lower limb index. Figure 4 presents 12 of the individual curves obtained for change in lower limb index between 5 and 11 years of age. As with the components of the index, findings will be drawn with reference to general course or trend, magnitude of change, and ranking in the group.

For 44 of the boys (63 per cent) the index declines continuously from 5 years to 11 years. In 30 instances the year-to-year decline gets progressively smaller, so that the trends classify as negatively accelerated decreasing functions of age. The first panel of Figure 3 shows four examples from this series. Curves P, U and Z of Figure 3 illustrate the 14 trends where there is ongoing descent, but not along a course of sustained convexity to the base line.

The index curves for 22 boys (31 per cent) descend between 7 and 9 years of age, with 10 staying practically on a level thereafter, and 12 turning from descent to ascent. Trend E of Figure 3 typifies those which fall to a plateau, and trend S those which follow a spread-U

course. Of the 4 curves remaining, one approximates a plateau from 5 to 8 years and then declines (Figure 3, F), one descends and ascends in a strongly convexo-concave course (Figure 3, T), and 2 rise slowly from 5 to 8 years and more rapidly from 8 to 11 years (Figure 3, M).

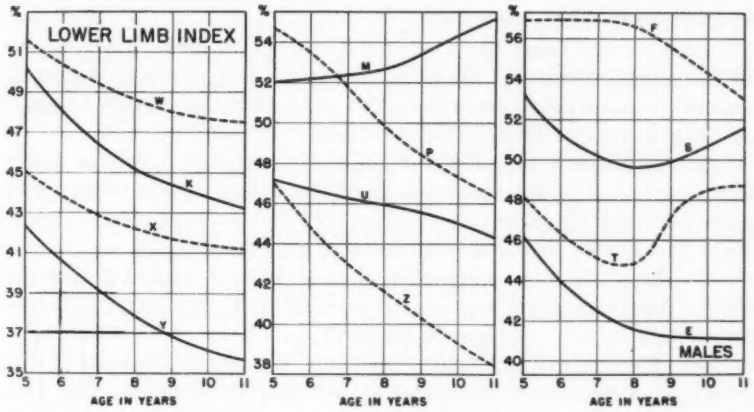

FIGURE 4

Twelve individual growth curves for leg girth in percentage of lower limb length.

The extremes of the distribution for change in lower limb index between ages 5 and 11 years are —9.2 and +3.1. Both of these changes are depicted in the middle panel of Figure 3, boy Z's index declining from 47.0 per cent to 37.8 per cent, and boy M's rising from 52.0 per cent to 55.1 per cent. The 10th and 90th percentiles of the distribution are —7.0 and —1.8, represented in Figure 3 by boys K and S respectively.

Over the age period 5 to 8 years, the lower limbs of 66 boys (94 per cent of those studied) become more slender; and over the age period 8 to 11 years, the lower limbs of 45 boys, or 64 per cent, become more slender. From these subgroups, Figure 3 includes the smallest decline between 5 and 8 years (boy U) and the largest decline between 8 and 11 years (boy Z). Also shown in Figure 3 are the largest rises in the index between 5 and 8 years (boy M) and between 8 and 11 years (boy T).

Summary

Three morphologic variables are investigated for a 6-year segment of the life cycle of the human male. The subjects are 70 nonpathologic white boys each measured at annual intervals throughout the postnatal period from age 5 years to age 11 years. It is shown that over the years of data accumulation (1940-54) reliability standards were maintained at a high level.

Numerous problems are considered, many of them necessitating seriatim measurement of the same organism. Use is made of central tendency, variability, correlation, and time series methods of analysis. Representative findings are as follows:

1. Between 5 and 11 years of age, the mean trend for lower limb length is a negatively accelerated increasing function of age. The mean trend for leg girth in percentage of lower limb length is a negatively accelerated decreasing function of age.

2. In relation to comparable samples of boys studied during the 1890's, the 11-year-old boys of the present study are larger in lower limb length by 5.0 cm., and in leg girth by 3.0 cm.

3. For lower limb length, both age and annual increment distributions approximate symmetrical bell-shaped surfaces. The corresponding frequency distributions for leg circumference manifest positive skewness.

4. Associations between size at age 5 years and gain from 5 to 11 years are $r = 0.5$ for lower limb length and $r = 0.4$ for leg girth. Eta coefficients are 0.6 for regression of (a) lower limb length on leg girth at age 5 years and (b) gain in lower limb length on gain in leg girth over the period 5 to 11 years.

5. Individual growth curves for lower limb length and leg girth are rising trends; those for leg girth in percentage of lower limb length mainly falling trends. Ninety per cent of the limb length trends are negatively accelerated functions of age, with the remainder approximating rectilinearity. Leg girth trends range from negative to positive curvilinearity, with a few showing first positive and then negative acceleration.

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INTERACTIONS BETWEEN STH AND CORTICOIDS IN THE REGULATION OF SOMATIC GROWTH, INFLAMMATION AND LYMPHATIC-TISSUE DEVELOPMENT[1]

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(Received for publication June 10, 1955)

Earlier experiments on rats had shown that the inhibition of somatic growth, inflammation and lymphatic tissue development by glucocorticoids (cortisone, cortisol)[2] can be antagonized by simultaneous treatment with the mineralocorticoid hormones (desoxycorticosterone, aldosterone). These mutual antagonisms are most readily demonstrable in adrenalectomized animals; normally the adrenals exert a "buffering effect" upon either type of exogenously administered corticoid, through the endogenous secretion of its natural antagonists (2, 3). Recent experiments have shown that, to some extent, even the ovaries can exert such a buffering action, presumably through the production of estradiol and progesterone (7); hence the ideal test object proved to be the adrenalectomized and ovariectomized rat.

The "growth hormone" or somatotrophic hormone (STH) tends to elicit effects which simulate those of the prophlogistic corticoids in many respects. For instance, in intact rats, it enhances the development of inflammatory reactions, as well as the growth of lymphatic tissue (thymus, lymph nodes, spleen). Of course, it also stimulates somatic growth but, in this respect, it differs from the prophlogistic corticoids; the latter can only prevent the inhibition of growth (normally caused by antiphlogistic corticoids) and thus restore a normal growth-rate, while STH actually enhances growth beyond the normal limits.

[1] These experiments were performed with the aid of a grant from the Defence Research Board, DR B C9310-35, Department of National Defence, Canada.

[2] In agreement with the suggestion made by Shoppee (9), the term cortisol is used here in preference to "hydrocortisone," since it eliminates confusion with 4,5-dihydrocortisone and obviates the possible implication that "hydrocortisone" is to be regarded primarily as a mere derivative of cortisone.

It is particularly noteworthy, however, that all the above mentioned actions of STH (stimulation of somatic growth, inflammation and lymphatic-tissue growth) depend upon the availability of prophlogistic corticoids. All these effects are readily demonstrable in intact rats as well as in adrenalectomized animals given maintenance doses of a prophlogistic corticoid, such as desoxycorticosterone (4) or aldosterone (5); they are not demonstrable, however, in adrenalectomized rats maintained exclusively on antiphlogistic corticoids, such as cortisone or cortisol. This "conditioning effect" upon STH of the mineralocorticoids (or "prophlogistic" corticoids), is apparently related to their influence on electrolyte metabolism since even maintenance on excess NaCl-supplements suffices to permit a certain degree of STH-responsiveness in the absence of the adrenals (6).

It is the object of this communication to report upon a series of experiments in which these manifold interactions between STH and the corticoids have been subjected to systematic study. In evaluating our data, it is important to keep in mind that the manifest end-result of a simultaneous treatment with antagonistic hormones is generally governed by the "law of the intersecting dose-effect curves" (8). With increasing doses, the actions of some hormones (e.g. desoxycorticosterone, aldosterone) rise rapidly and then reach a plateau over which a further increase in effect cannot be obtained by raising the dose; the actions of other hormones (e.g., cortisol) do not rise as steeply with an increase in dosage, but continue to become more and more pronounced until near-fatal doses are reached. Consequently, at low dose-levels the former, at high dose-levels the latter, of these opposing hormone-effects will predominate.

For instance, it is possible to prepare a mixture, containing desoxycorticosterone acetate (DOCA) and cortisol acetate (COLA), in fixed proportions, of which small amounts stimulate, while large amounts inhibit anabolism, inflammation and lymphatic-tissue development (8).

All these factors had to be taken into consideration in designing systematic experiments to explore the interrelations between STH and the two opposing types of corticoids. Consequently, it was necessary not only to determine the effect of every one of these hormones alone and in combination, but also, that of varying the dose-level of each substance in animals receiving treatment with several hormones at the same time.

Materials and Techniques

General experimental design.—In agreement with the considerations outlined in our introduction, three series of experiments were necessary to explore the interactions between STH and the two types of corticoids, desoxycorticosterone acetate (DOCA) and cortisol acetate (COLA).

Series I	*Series II*	*Series III*
Experiments 1-9.—Ascending doses of STH in combination with unchanging or ascending doses of the other hormones:	*Experiments 10-18.*—Ascending doses of DOCA in combination with unchanging or ascending doses of the other hormones:	*Experiment 19-27.*—Ascending doses of COLA in combination with unchanging or ascending doses of the other hormones:
(1) STH (ascending doses)	(10) DOCA (ascending doses)	(19) COLA (ascending doses)
(2) STH (ascending doses) +DOCA (medium dose)	(11) DOCA (ascending doses) +STH (medium dose)	(20) COLA (ascending doses) +STH (medium dose)
(3) STH (ascending doses) +COLA (medium dose)	(12) DOCA (ascending doses) +COLA (medium dose)	(21) COLA (ascending doses) +DOCA (medium dose)
(4) STH (ascending doses) +DOCA (medium dose) +COLA (medium dose)	(13) DOCA (ascending doses) +STH (medium dose) +COLA (medium dose)	(22) COLA (ascending doses) +STH (medium dose) +DOCA (medium dose)
(5) STH (ascending doses) +DOCA (ascending doses)	(14) DOCA (ascending doses) +STH (ascending doses) (*Same as "5"*)	(23) COLA (ascending doses) +STH (ascending doses) (*Same as "7"*)
(6) STH (ascending doses) +DOCA (ascending doses) +COLA (medium dose)	(15) DOCA (ascending doses) +STH (ascending doses) +COLA (medium dose) doses) (*Same as "6"*)	(24) COLA (ascending doses) +STH (ascending doses) +DOCA (medium dose) (*Same as "8"*)
(7) STH (ascending doses) +COLA (ascending doses)	(16) DOCA (ascending doses) +COLA (ascending doses)	(25) COLA (ascending doses) +DOCA (ascending doses) (*Same as "16"*)
(8) STH (ascending doses) +DOCA (medium dose) +COLA (ascending doses)	(17) DOCA (ascending doses) +STH (medium dose) +COLA (ascending doses)	(26) COLA (ascending doses) +STH (medium dose) +DOCA (ascending doses) (*Same as "17"*)
(9) STH (ascending doses) +DOCA (ascending doses) +COLA (ascending doses)	(18) DOCA (ascending doses) +STH (ascending doses) +COLA (ascending doses) (*Same as "9"*)	(27) COLA (ascending doses) +STH (ascending doses) +DOCA (ascending doses) (*Same as "9"*)

It will be noted, however, that some of the experiments in the three series are actually identical (e.g., Experiments 5 and 14 or Experiments 9, 18 and 27). Hence, here in discussing each series, we merely have to use cross-references. Furthermore, some of these hormone-combinations have already been adequately assayed and published in earlier work (e.g., Experiments 10 or 21) and here, reference to the literature will suffice. Nevertheless it was felt that, for a synoptic presentation of the entire topic, the material should be presented in the form of these three series, giving data on all the hormone combinations conjointly.

Conversely, in some instances, additional experiments were considered to be informative. For instance, to compare the response of the adrenalectomized and ovariectomized animals with that of intact controls, or to compare animals maintained on water with those receiving NaCl-supplements. Data on these are also presented.

Experimental animals.—In order to ensure uniformity, for all the experiments to be reported here, only female Sprague-Dawley rats, weighing 150-170 gm. at the onset of the experiment, were used. The animals were subdivided into groups consisting of ten rats each and having an average body-weight of no less than 158 gm. and no more than 162 gm.

Preparation of the "granuloma-pouch" for the study of inflammation.—In all experimental animals, a granuloma-pouch was prepared for the objective quantitative assessment of inflammation. The details of this procedure have been described elsewhere (8). For the purpose of the present experimental series, 25 ml. of air was injected under the shaved dorsal skin of the rat, under ether anesthesia, this being immediately followed by the injection of 0.5 ml. of 1% croton oil (in corn oil) into the air-space so created.

Adrenalectomy and ovariectomy.—With the exception of a few control experiments on intact or merely adrenalectomized rats (specifically so designated in the text), all animals were adrenalectomized and ovariectomized 48 hours after the preparation of the granuloma-pouch. The operation was performed in a single session under anesthesia, through two separate subcostal incisions, placed just lateral from the margin of the granuloma-pouch on each side.

Administration of hormones.—All hormone preparations were injected once daily subcutaneously (in the ventral and inguinal regions), in 0.2 ml. of water. COLA [hydrocortisone acetate ("Cortril,"

Pfizer)] and DOCA [desoxycorticosterone acetate ("Cortate," Schering)] were given in the form of microcrystal suspensions. STH [somatotrophic hormone ("Somatotropin," Armour)] was administered as a solution in the same amount of water, although with the high concentrations, part of the material was in the form of a suspension. The individual daily dose of cortisol, DOCA and STH varied between 50 μg. and 2000 μg., as indicated in our tables. Treatment with all hormones commenced on the day of adrenalectomy.

Diet.—All animals were kept exclusively on "Purina Fox Chow." As a drinking fluid, they received either tap water or a 1% NaCl solution, as described in the text.

Indicators of activity.—All animals were killed 12 days after preparation of the granuloma-pouch, that is, ten days after removal of their steroid-producing endocrine glands and the initiation of hormone treatment.

Immediately after death the exudate was removed, measured in a graduate cylinder, then the body-weight of the animals was determined (without exudate). Subsequently the thymus and spleen were dissected, fixed in formalin and weighed on an analytical balance. The final *body-weight,* the volume of *exudate* and the weight of the *thymus* and *spleen* were taken as indicators of somatic growth, inflammatory potential and lymphatic-tissue development. The means of these determinations (followed by the standard errors for statistical evaluation), as well as the percentual gain (or loss) in somatic weight and the percentual mortality during the experiment are listed in our tables.

Results

Series I—Ascending doses of STH

(1) *STH (ascending doses).*—Previous experiments had amply demonstrated that STH is highly toxic to adrenalectomized-ovariectomized rats. Even merely adrenalectomized animals cannot withstand treatment with growth-promoting doses of this hormone, unless they are simultaneously given at least maintenance amounts of corticoids or NaCl supplements (4, 8). Consequently, we attempted to determine the effect of ascending doses of STH only in adrenalectomized rats given 1% NaCl as a drinking fluid for maintenance. Ovariectomy generally further decreases the resistance of adrenalectomized rats (8) by removing the source of progesterone (a steroid endowed with

definite corticoid properties) and hence, in this experimental arrangement, it was thought preferable to refrain from further aggravating the condition of the animals by a simultaneous gonadectomy. The results of these experiments are summarized in Table 1.

It will be noted that with NaCl-supplements as a maintenance therapy, STH stimulated somatic growth in comparison with the adrenalectomized controls receiving no hormone treatment (Group II), but it failed to raise the final body-weight above that of the sham adrenalectomized controls (Group I).

There was no clearcut effect upon exudate-formation. The thymus is notoriously much larger in adrenalectomized (Group II) than in intact (Group I) rats, but STH caused little if any change from the control level after adrenalectomy. The comparatively small spleen of the adrenalectomized rat tended to be restored towards normal with ascending doses of STH. However, in view of the relatively large standard error of the variable lymphatic organs, these changes in thymic and splenic weight are of doubtful significance.

It is also noteworthy that, as judged by the mortality rate, at all dose-levels above 500 μg/day, STH was definitely toxic to these adrenalectomized rats, despite NaCl maintenance therapy. Yet, NaCl can be taken as having improved resistance to STH, since previous experiments had shown that adrenalectomized rats maintained on tap water invariably die when treated with 1 or 2 mg./STH/day (100% mortality with 2 mg.) (4).

(2) *STH* (*ascending doses*) + *DOCA* (*medium dose*).—This experiment was performed with the routine technique on adrenalectomized and ovariectomized animals given only tap water to drink. The results are summarized in Table 2.

For this type of experiment it is technically not possible to prepare satisfactory untreated controls, since after simultaneous adrenalectomy and ovariectomy, rats do not survive in the absence of corticoid treatment if they are not given salt supplements. It may be said, however, that here—as in the NaCl-treated-adrenalectomized rats of Experiment 1—STH increased the body-weight in proportion to the amount of hormone given, but only up to the normal level. Exudate-formation was also raised by STH up to the level of the intact controls (Group I in Experiment 1) but no further. There was no significant change in the thymic and splenic weights, but all doses of STH were well tolerated without mortality.

TABLE 1
(Experimental arrangement No. 1)
Effect of STH (ascending doses) on adrenalectomized rats maintained on 1% NaCl.

Group	*Treatment* STH (μg.)	Final Body-weight (mg.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	—*	186 ± 3.4	+ 17.0	18.0 ± 1.1	282 ± 17.8	1042 ± 83	0
II	—**	167 ± 4.7	+ 3.8	12.8 ± 1.9	426 ± 23.5	715 ± 51	0
III	50	173 ± 5.6	+ 7.8	15.3 ± 1.3	360 ± 24.0	797 ± 50	0
IV	100	176 ± 4.8	+ 9.8	13.6 ± 1.4	342 ± 21.4	924 ± 113	0
V	500	175 ± 2.7	+ 9.0	14.5 ± 2.3	321 ± 61.0	621 ± 61	50
VI	1000	177 ± 6.4	+ 11.5	11.7 ± 1.4	364 ± 31.0	890 ± 69	10
VII	5000	190 ± 4.4	+ 19.8	10.6 ± 0.9	471 ± 46.0	1056 ± 204	50

* Sham—adrenalectomized controls.
** Adrenalectomized controls.

TABLE 2
(Experimental arrangement No. 2)
Effect of STH (ascending doses) + DOCA (medium dose) on adrenalectomized and ovariectomized rats.

Group	*Treatment* STH (μg.)	DOCA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	100	175 ± 3.0	+ 6.7	12 ± 2.1	386 ± 21.0	706 ± 36.2	0
II	100	100	177 ± 3.2	+ 8.5	14 + 2.1	394 ± 24.0	768 ± 45.0	0
III	600	100	186 ± 3.7	+ 14.8	17 ± 0.7	415 ± 33.0	791 ± 30.6	0
IV	2000	100	191 ± 2.5	+ 18.0	17 ± 1.7	377 ± 21.9	895 ± 61.5	0

(3) *STH* (*ascending doses*) + *COLA* (*medium dose*).—This experiment was performed on ten groups of rats—five kept on water and five on 1% NaCl solution as a drinking fluid—in order to determine clearly the role of sodium as a conditioning factor of STH, in rats deprived of both their steroid producing glands and maintained on a glucocorticoid alone. The results of both these series are listed in Table 3.

In the animals maintained on water (Groups I-IV), STH proved to be singularly ineffective in every respect, in comparison with its activity in the animals given NaCl supplements. On tap water, the enormous dose of 3000 μg. of STH/day (Group V) was required to antagonize COLA, as well as 1% NaCl did without STH (Group VI). It is as though the whole baseline for the response to STH were raised by the salt supplement. Thus only combined treatment with NaCl and STH, resulted in an almost complete restoration of somatic, thymic and splenic weights to the values seen in intact controls (Table 1, Group I); even here exudation remained somewhat subnormal.

(4) *STH* (*ascending doses*) + *DOCA* (*medium dose*) + *COLA* (*medium dose*).—This experiment was performed under standard conditions on adrenalectomized and ovariectomized rats maintained on water (Table 4).

As we shall see later (Experimental arrangement No. 12) at this dose-level, DOCA alone suffices to counteract the growth-inhibitory, antiphlogistic and lympholytic effects of cortisol to approximately normal values. Hence it is not unexpected that even Group I of the present experimental series, which was treated with as little as 50 μg. of STH/day, exhibited essentially normal values. However, by further raising the dose of STH its effects were only slightly (and in most instances insignificantly) augmented, except as regards somatic growth itself.

(5) *STH* (*ascending doses*) + *DOCA* (*ascending doses*).—This experiment was again performed under standard conditions on adrenalectomized and ovariectomized rats (Table 5).

Here, the results were almost the same as those of Experiment No. 2 in which the effect of ascending doses of STH were tested in rats receiving a medium dose of DOCA. However, somatic weight, as well as the development of the thymus and spleen, were somewhat

TABLE 3
(Experimental arrangement No. 3)
Effect of STH (ascending doses) + COLA (medium dose) on adrenalectomized and ovariectomized rats maintained on water or 1% NaCl.

Group	*Treatment* STH (μg.)	COLA (μg.)	Drinking Fluid	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	—	400	Water	135 ± 3.2	— 16.1	5.0 ± 0.9	134 ± 20.5	624 ± 77.9	0
II	100	400	Water	138 ± 4.9	— 14.2	5.0 ± 0.4	148 ± 22.5	676 ± 49.2	0
III	500	400	Water	148 ± 2.5	— 7.9	7.8 ± 1.3	160 ± 13.3	508 ± 44.5	0
IV	1000	400	Water	153 ± 6.0	— 6.1	6.5 ± 1.9	171 ± 21.3	721 ± 100.0	0
V	3000	400	Water	161 ± 2.4	+ 0.6	6.4 ± 1.5	215 ± 18.6	723 ± 71.0	0
VI	—	400	NaCl	159 ± 2.5	— 1.8	7.5 ± 1.4	198 ± 20.4	792 ± 57.8	0
VII	100	400	NaCl	157 ± 2.9	— 3.0	13.0 ± 3.1	220 ± 19.7	902 ± 98.3	0
VIII	500	400	NaCl	165 ± 3.0	+ 2.4	12.0 ± 1.7	280 ± 15.3	824 ± 96.0	0
IX	1000	400	NaCl	177 ± 2.8	+ 10.0	9.0 ± 1.6	259 ± 20.4	785 ± 61.5	0
X	3000	400	NaCl	184 ± 3.1	+ 14.2	11.0 ± 2.1	296 ± 14.3	904 ± 103.8	0

TABLE 4
(Experimental arrangement No. 4)
Effect of STH (ascending doses) + DOCA (medium dose) + COLA (medium dose) on adrenalectomized and ovariectomized rats.

Group	*Treatment* STH (μg.)	DOCA (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	100	400	171 ± 1.7	+ 6.2	14.6 ± 1.8	318 ± 24.3	694 ± 40.7	0
II	100	100	400	165 ± 2.5	+ 3.1	15.5 ± 2.8	240 ± 17.8	685 ± 55.5	0
III	600	100	400	176 ± 1.8	+ 10.0	13.7 ± 2.1	337 ± 38.8	737 ± 46.0	0
IV	2000	100	400	181 ± 2.7	+ 12.1	16.5 ± 1.5	308 ± 18.9	721 ± 58.2	0

more markedly and more consistently raised by STH in the presence of a great excess of DOCA.

(6) *STH (ascending doses) + DOCA (ascending doses) + COLA (medium dose).*—This test was also performed under standard conditions on adrenalectomized and ovariectomized rats (Table 6).

It will be noted that this experiment differs from the previous one only in that all groups received 400 μg./day of cortisol. Interestingly, this small amount of glucocorticoid—it is just sufficient to restore an approximately normal resistance to an adrenalectomized rat—virtually nullified the strong somatotrophic prophlogistic, thymotrophic and splenotrophic actions of combined treatment with STH and DOCA.

(7) *STH (ascending doses) + COLA (ascending doses).*—This experiment was performed under standard conditions on adrenalectomized and ovariectomized rats (Table 7).

If we compare these results with those obtained in Experiment No. 3, in which ascending doses of STH were administered simultaneously with a medium dose of cortisol to adrenalectomized and ovariectomized animals maintained on water (Table 3, Groups I-V), it will be noted that in both these series, STH failed to influence markedly any of the indicators studied. We are inclined to attribute this to the absence of DOCA and of dietary NaCl supplements. The involution of the thymus and spleen following treatment with 2000 μg. of COLA/day (Table 7, Group IV) is virtually maximal and we may conclude that, in these respects at least, STH was totally ineffective in antagonizing cortisol. On the other hand, the catabolic and antiexudative actions of such high doses of cortisol would be much more intense in the absence of STH treatment (compare with experimental arrangement No. 19) so that in this respect apparently STH does exert a slight anticortisol effect under the present experimental conditions.

(8) *STH (ascending doses) + DOCA (medium dose) + COLA (ascending doses).*—This experiment was also performed under standard conditions in adrenalectomized and ovariectomized rats, but another control group was added in which rats subjected to sham adrenalectomy and ovariectomy (not receiving any hormone therapy) were used for comparison (Table 8).

It is especially instructive to compare the results of this experiment with those of the previous one from which it differs only in additional

TABLE 5
(Experimental arrangement No. 5)
Effect of STH (ascending doses) + DOCA (ascending doses) on adrenalectomized and ovariectomized rats.

Group	*Treatment* STH (μg.)	DOCA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	50	161 ± 4.7	+ 0.6	16 ± 1.8	446 ± 28.1	779 ± 87.5	20
II	100	100	178 ± 2.6	+ 11.2	16 ± 1.5	522 ± 27.2	855 ± 54.3	0
III	600	400	190 ± 3.8	+ 18.7	18 ± 2.5	489 ± 35.3	1047 ± 59.7	0
IV	200	2000	205 ± 3.4	+ 28.1	17 ± 1.5	496 ± 30.2	1286 ± 87.9	0

TABLE 6
(Experimental arrangement No. 6)
Effect of STH (ascending doses) + DOCA (ascending doses) + COLA (medium dose) on adrenalectomized and ovariectomized rats.

Group	*Treatment* STH (μg.)	DOCA (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	50	400	163 ± 3.8	+ 1.2	7.0 ± 1.5	202 ± 25.0	582 ± 30.9	0
II	100	100	400	165 ± 1.5	+ 3.0	10.0 ± 1.4	196 ± 14.6	701 ± 84.0	0
III	600	400	400	174 ± 1.1	+ 8.7	8.0 ± 1.3	205 ± 11.9	641 ± 56.5	0
IV	2000	2000	400	170 ± 2.7	+ 6.2	6.0 ± 1.3	176 ± 13.6	579 ± 13.0	0

TABLE 7
(Experimental arrangement No. 7)
Effect of STH (ascending doses) + COLA (ascending doses) on adrenalectomized and ovariectomized rats.

Group	*Treatment* STH (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	50	147 ± 5.4	—8.7	7.0 ± 1.4	265 ± 26.4	437 ± 69.8	10
II	100	100	155 ± 2.7	—2.0	10.0 ± 1.4	359 ± 24.2	600 ± 36.4	20
III	600	400	160 ± 2.0	—0.6	7.0 ± 1.3	168 ± 20.2	450 ± 44.3	10
IV	2000	2000	150 ± 2.1	—6.8	4.0 ± 1.2	12 ± 7.3	365 ± 13.3	10

TABLE 8
(Experimental arrangement No. 8)
Effect of STH (ascending doses) + DOCA (medium dose) + COLA (ascending doses) on adrenalectomized and ovariectomized rats.

Group	*Treatment* STH (μg.)	DOCA (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	—*	—*	—*	180 ± 3.1	+ 11.1	13 ± 1.6	211 ± 20.6	849 ± 86.2	0
II	50	100	50	173 ± 4.5	+ 8.1	18 ± 1.5	402 ± 33.1	965 ± 65.7	0
III	100	100	100	176 ± 1.8	+ 10.0	16 ± 3.1	322 ± 31.6	968 ± 66.2	0
IV	600	100	400	174 ± 5.2	+ 8.7	17 ± 2.3	232 ± 12.3	886 ± 50.9	0
V	2000	100	2000	158 ± 2.1	— 2.4	6 ± 1.6	34 ± 2.2	421 ± 28.6	0

* Sham-adrenalectomized and sham-ovariectomized, untreated controls.

treatment with medium doses of DOCA. It will be noted that this mineralocorticoid markedly stimulated somatic growth, exudation, thymic development and splenic growth, when low or medium doses of the other two hormones were given (compare Groups II, III and IV of Table 8 with Groups I, II, III of Table 7). However, at the dose of 2000 μg./day of cortisol, the catabolic antiphlogistic, thymolytic and splenolytic effects of this hormone were virtually uninhibited by the combined treatment with DOCA and STH.

(9) *STH (ascending doses) + DOCA (ascending doses) + COLA (ascending doses).*—This experiment was performed under standard conditions in adrenalectomized and ovariectomized rats, but here again, an additional group of sham adrenalectomized and ovariectomized untreated controls was also included (Table 9).

It will be noted that here again, as in the two preceding experiments, body-weight, exudation, as well as thymic and splenic development were inversely proportional to the dose of cortisol given, despite the simultaneous treatment with very high doses of STH and DOCA.

Series II—Ascending Doses of DOCA

(10) *DOCA (ascending doses).*—Since a large number of experiments of this type had been carried out previously under essentially similar circumstances (8), it will suffice to mention here that at the dose of 100μg./day, DOCA sufficed to produce maximal stimulation of somatic weight increase, exudation, as well as thymic and splenic development in otherwise untreated rats. By further raising the dose of DOCA (without additional STH-treatment), none of these effects could be raised significantly above the level they had attained on 100 μg./day, although tests were made up to the level of 5000 μg./day. In fact, these were the findings which led us to the conclusion that treatment with a mineralocorticoid rapidly reaches a "plateau of effectiveness" in all these respects.

Since it is of great importance for the concept of corticoid-antagonisms to establish the effect of ascending doses of DOCA under various conditions, complementary experiments have been carried out however on adrenalectomized and ovariectomized rats under otherwise standard conditions, but given NaCl as drinking fluid (Table 10-A).

The main outcome of this work was to show that, on adrenalecto-

TABLE 9
(Experimental arrangement No. 9)
Effect of STH (ascending doses) + DOCA (ascending doses) + COLA (ascending doses) on adrenalectomized and ovariectomized rats.

Group	*Treatment* STH (μg.)	DOCA (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	—*	—*	—*	183 ± 1.7	+ 12.9	9 ± 1.3	246 ± 17.5	841 ± 105.2	0
II	50	50	50	178 ± 3.6	+ 9.8	12 ± 1.0	442 ± 18.6	1193 ± 137.2	0
III	100	100	100	178 ± 4.2	+ 10.5	12 ± 1.6	393 ± 31.9	869 ± 67.9	0
IV	600	400	400	179 ± 2.8	+ 11.1	9 ± 0.3	216 ± 16.5	850 ± 134.1	0
V	2000	2000	2000	162 ± 2.2	+ 1.2	4 ± 1.1	33 ± 7.1	588 ± 126.9	0

* Sham-adrenalectomized and sham-ovariectomized, untreated controls.

mized and ovariectomized rats maintained on 1% NaCl, even 50 μg. of DOCA/day suffices to produce virtually maximal effects upon all the indicators under investigation.

Additional observations with this experimental arrangement have also been performed on intact females in which the granuloma-pouch was made 48 hours before the initiation of hormone treatment, as in all standard experiments, but the ovaries and adrenals were not removed (Table 10-B).

Then we repeated the experiment in intact females in which hormone treatment was initiated on the day on which the granuloma-pouch was prepared Table 10-C).

In all these intact females—whether hormone treatment was initiated 48 hours after or simultaneously with the preparation of the granuloma-pouch—DOCA did not exert any noticeable effect under these experimental conditions. Of course, it could not have shown its efficacy by life-maintenance (all these intact animals would have survived anyway) but it is noteworthy that there was no stimulation of somatic growth or exudation and only a slight and probably insignificant increase in splenic weight as compared, for instance, with the sham-adrenalectomized and sham-ovariectomized controls of Table 9. This may well be due to the fact that the normal functioning adrenal produces the equivalent of about 100 μg./day of mineralocorticoid activity, anyway, so that an increase above this level does not result in a further augmentation of the effect. It is difficult to understand, however, why the mean thymus weight was not further increased. Presumably, the endogenous glucocorticoids are more efficient in causing thymus atrophy in the presence of optimal concentrations of mineralocorticoids than they are in inhibiting growth or exudation.

Finally, we gave ascending doses of DOCA to adrenalectomized but not gonadectomized male rats under otherwise standard conditions (Table 10-D).

Adrenalectomized male Sprague-Dawley rats have singularly high requirements for corticoid substitution-therapy. (That is why we preferred adrenalectomized and ovariectomized females for routine experiments on the effects of corticoids in the absence of adrenal and ovarian hormones.) This great sensitivity is quite evident from the results of the present experimental series, since it was necessary to administer as much as 400 μg./day of DOCA for maintenance. On the 100 μg./day dose-level, there still was 20% mortality although, in

TABLE 10-A
(Experimental arrangement No. 10)
Effect of DOCA (ascending doses) on adrenalectomized and ovariectomized rats maintained on 1% NaCl.

Group	*Treatment* DOCA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	176 ± 3.6	+ 10.0	16 ± 0.6	441 ± 28	722 ± 55.0	10
II	100	183 ± 2.4	+ 13.6	14 ± 2.1	436 ± 28	716 ± 28.6	0
III	400	180 ± 2.6	+ 12.5	15 ± 1.7	357 ± 42	801 ± 24.6	0
IV	2000	183 ± 3.1	+ 13.0	15 ± 1.7	442 ± 40	909 ± 53.2	0

TABLE 10-B
(Experimental arrangement No. 10)
Effect of DOCA (ascending doses) on intact female rats in which hormone treatment was initiated 48 hours after preparation of the granuloma-pouch.

Group	*Treatment* DOCA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	100	180 ± 1.7	+ 12.5	16 ± 1.3	268 ± 23.3	902 ± 44.2	0
II	400	176 ± 1.7	+ 10.0	17 ± 2.4	242 ± 24.1	1091 ± 94.2	0
III	1000	176 ± 1.4	+ 10.0	17 ± 2.0	261 ± 13.7	1156 ± 83.2	0
IV	5000	176 ± 2.6	+ 10.0	14 ± 1.4	302 ± 24.0	1103 ± 69.7	0

TABLE 10-C
(Experimental arrangement No. 10)
Effect of DOCA (ascending doses) on intact female rats in which hormone treatment was initiated on the day on which the granuloma-pouch was prepared.

Group	*Treatment* DOCA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	100	171 ± 3.0	+ 6.9	17 ± 1.8	238 ± 22.1	1012 ± 78.5	0
II	400	181 ± 2.2	+ 13.1	14 ± 2.2	281 ± 28.1	1156 ± 99.5	0
III	1000	172 ± 3.7	+ 7.5	15 ± 2.9	279 ± 16.7	1148 ± 74.4	0
IV	5000	176 ± 3.2	+ 10.7	18 ± 1.7	282 ± 30.1	1168 ± 98.0	0

TABLE 10-D
(Experimental arrangement No. 10)
Effect of DOCA (ascending doses) on adrenalectomized but not gonadectomized male rats.

Group	*Treatment* DOCA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	178 —	+ 3.5	7 —	381 —	656 —	80
II	100	187 ± 3.9	+ 16.1	20 ± 3.3	444 ± 32.9	1148 ± 89.1	20
III	400	192 ± 3.5	+ 18.5	17 ± 3.2	458 ± 31.4	1217 ± 121.0	0
IV	1000	189 —	+ 11.1	18 —	439 —	1188 —	0

the surviving animals, the indicators of activity were maximally stimulated.

(11) *DOCA (ascending doses) + STH (medium dose).*—This experiment was performed under standard experimental conditions, on adrenalectomized and ovariectomized rats (Table 11).

It appears that a medium dose of 600 μg./day of STH resulted in little if any augmentation of the DOCA-effects at any dose-level of the latter hormone.

(12) *DOCA (ascending doses) + COLA (medium dose).*—The results of this type of experiment have also been adequately described elsewhere (8). In essence, it has been found that the effects of medium doses of cortisol, upon growth, inflammation and thymolysis, are adequately antagonized by as little as 100 μg/day of DOCA. Further raising the dose of the latter hormone does not result in a more efficient neutralization of the glucocorticoid.

This may be the explanation of the apparently paradoxical findings reported above in connection with experimental arrangement No. 10, namely, that in the presence of the adrenals (which presumably produce adequate amounts of both kinds of corticoids) even additional treatment with DOCA does not completely antagonize the effects (especially the thymolytic effect) of the endogenous glucocorticoids. It will be recalled that the thymus of DOCA-treated, intact rats was always much smaller than that of adrenalectomized DOCA-treated animals.

Now the question arose, whether this apparent ineffectiveness of DOCA could be due to the fact that the hormone is optimally effective only when growth exudation and thymicolymphatic development are very intensely inhibited. In intact rats, as well as in adrenalectomized-ovariectomized animals receiving only maintenance doses of cortisol, the indicators of DOCA activity are normal or only slightly below the normal level. Therefore, it was considered appropriate to perform one experiment on intact females, in which marked inhibition of somatic growth, exudation and thymicolymphatic development had been produced by treatment with comparatively *large doses (1000 μg/day) of cortisol* (Table 12).

It will be noted that although here 1000 μg/day of cortisol sufficed to inhibit somatic growth and exudation to a considerable extent, and to produce very pronounced thymic and splenic atrophy, DOCA nevertheless failed to restore the targets of activity to their normal level. This shows quite clearly that the relative inefficacy of DOCA,

TABLE 11
(Experimental arrangement No. 11)
Effect of DOCA (ascending doses) + STH (medium dose) on adrenalectomized and ovariectomized rats.

Group	*Treatment* DOCA (μg.)	STH (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	600	177 ± 3.4	+ 10.6	11.0 ± 1.8	409 ± 28.2	806 ± 77.4	10
II	100	600	181 ± 2.8	+ 14.5	16.0 ± 1.7	458 ± 19.6	1126 ± 146.3	0
III	400	600	192 ± 3.7	+ 20.0	18.0 ± 1.9	415 ± 31.0	1405 ± 86.6	0
IV	2000	600	197 ± 2.6	+ 23.1	18.0 ± 3.1	470 ± 15.1	1169 ± 124.5	0

TABLE 12
(Experimental arrangement No. 12)
Effect of DOCA (ascending doses) + COLA (1000 μg/day) on intact female rats .

Group	*Treatment* DOCA (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	—	1000	154 ± 1.7	—3.8	9.0 ± 0.8	49 ± 5.3	494 ± 77.5	0
II	100	1000	151 ± 2.8	—6.2	7.0 ± 1.3	64 ± 8.2	420 ± 31.5	0
III	1000	1000	158 ± 2.2	—1.3	9.0 ± 1.2	57 ± 9.2	417 ± 15.5	0
IV	5000	1000	152 ± 3.8	—5.0	6.0 ± 1.5	35 ± 3.4	447 ± 18.7	20

under such conditions, is inherent in the experimental arrangement—presumably because it is governed by the "law of intersecting dose-effect curves." It is certainly not merely due to an inability of DOCA to act unless these targets are far below normal, since here they were all greatly depressed and yet no dose of DOCA restored them towards normalcy.

(13) *DOCA (ascending doses) + STH (medium dose) + COLA (medium dose).*—This experiment was performed under standard conditions in adrenalectomized and ovariectomized rats (Table 13).

It is evident that even in the presence of maintenance-doses of cortisol, DOCA (at any dose-level) even in association with medium doses of STH, failed to restore optimally the somatic growth-rate, exudation and the development of the thymus and spleen.

(14) *DOCA (ascending doses) + STH (ascending doses).*—This arrangement is identical with that of Experiment 5 and hence requires no further discussion here (Table 5).

(15) *DOCA (ascending doses) + STH (ascending doses) + COLA (medium dose).*—This arrangement is identical with that of Experiment 6 (Table 6).

(16) *DOCA (ascending doses) + COLA (ascending doses).*—Experiments in which equal amounts of DOCA and COLA were administered simultaneously, at different dose-levels, between 10 μg. and 5000 μg. of each steroid, have been published elsewhere (8). At dose-levels below 50 μg. of each steroid per day, the results were quite variable and most of the animals died because substitution therapy was insufficient for normal life maintenance. However, at higher dose-levels, the results were entirely in agreement with the "law of the intersecting dose-effect curves." At the dose-levels of 50 and 100 μg/day of both steroids, the prophlogistic, anabolic and lymphatic-tissue stimulating effects of DOCA predominated. At about the level of 300 μg/day, the two steroids mutually neutralized each other and the targets were essentially in the same condition as in the not hormone-treated controls. At still higher dose-levels the antiphlogistic, catabolic and lympholytic actions of COLA predominated.

(17) *DOCA (ascending doses) + STH (medium dose) + COLA (ascending doses).*—This experiment was performed under standard conditions on adrenalectomized and ovariectomized rats (Table 14).

The results of these experiments were essentially similar to those obtained in the Experiment No. 16. At the 50 and 100 μg. level,

TABLE 13
(Experimental arrangement No. 13)
Effect of DOCA (ascending doses) + STH (medium dose) + COLA (medium dose) on adrenalectomized and ovariectomized rats.

	Treatment								
Group	DOCA (μg.)	STH (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	600	400	174 ± 3.7	+ 8.0	13 ± 2.4	178 ± 16.5	644 ± 68.7	0
II	100	600	400	172 ± 0.4	+ 7.5	12 ± 0.9	181 ± 12.1	550 ± 10.4	0
III	400	600	400	175 ± 1.9	+ 9.4	15 ± 1.6	186 ± 22.7	672 ± 42.1	0
IV	2000	600	400	174 ± 1.5	+ 8.0	11 ± 1.8	177 ± 14.2	736 ± 53.9	0

TABLE 14
(Experimental arrangement No. 17)
Effect of DOCA (ascending doses) + STH (medium dose) + COLA (ascending doses) on adrenalectomized and ovariectomized rats.

	Treatment								
Group	DOCA (μg.)	STH (μg.)	COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	600	50	170 ± 2.7	+ 6.2	12 ± 0.7	434 ± 30.1	797 ± 33.3	0
II	100	600	100	179 ± 3.4	+ 10.5	10 ± 1.8	461 ± 46.9	824 ± 29.4	0
III	400	600	400	176 ± 2.3	+ 9.3	11 ± 1.5	208 ± 14.5	674 ± 49.9	0
IV	2000	600	2000	147 ± 2.2	— 8.6	5 ± 1.4	33 ± 2.4	361 ± 17.3	0

combined treatment with the two steroids permitted adequate growth, exudation and lymphatic-tissue development. At the 400 μg/day level, the thymus and spleen began to show signs of involution, although somatic growth and exudation were still quite marked. At the 2000 μg/day level, the animals actually lost weight and exudation, as well as the involution of the thymus and spleen were virtually the same as if COLA alone had been given (compare with Table 15).

(18) *DOCA (ascending doses)* + *STH (ascending doses)* + COLA *(ascending doses)*.—This arrangement is identical with that of Experiment No. 9 (Table 9).

Series III—Ascending doses of COLA

(19) *COLA (ascending doses)*.—We had shown previously that, in rats kept on tap water, ascending doses of COLA produce increasingly more pronounced catabolism, inhibition of inflammation and lymphatic-tissue involution at all dose-levels in excess of 50 μg/day although, in most respects, virtually maximal effects are noted at the level 500 μg/day (8).

We have now repeated this work on *adrenalectomized* (not ovariectomized) *females, receiving 1% NaCl* as a drinking fluid, to verify whether here again NaCl would exhibit its usual anticortisol actions (Table 15-A).

It will be noted that, in agreement with expectations, here all the effects of COLA, which were subjected to study, proved to be less pronounced than if the animals had been maintained on tap water. Some degree of somatic growth was possible and exudation, as well as involution of the thymus and spleen were far from being maximally inhibited, even at the daily dose-level of 500 μg. Here, all the actions of COLA were increasingly more pronounced as the dose was raised between 100 μg. (the minimum dose required for substitution) and 5000 μg/day.

We then proceeded to repeat this type of experiment *in intact female rats under standard conditions* (Table 15-B).

It will be noted that here the presence of the adrenals inhibited the actions of cortisol approximately to the same extent as NaCl did in adrenalectomized animals (cf. Table 15-A). However, here again, there was a fairly close proportionality between dose and effects.

Essentially similar results were noted in *intact female rats in which*

TABLE 15-A
(Experimental arrangement No. 19)
Effect of COLA (ascending doses) on adrenalectomized rats maintained on 1% NaCl.

Group	*Treatment* COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	174 ± 4.1	+ 8.0	12.7 ± 1.4	309 ± 21.0	785 ± 45	0
II	100	172 ± 4.3	+ 7.6	19.8 ± 2.9	313 ± 25.0	1096 ± 116	10
III	500	166 ± 3.3	+ 3.3	10.5 ± 1.3	184 ± 18.0	643 ± 78	0
IV	1000	155 ± 2.6	— 3.2	8.7 ± 1.7	75 ± 5.6	716 ± 135	0
V	5000	136 ± 2.6	— 15.0	3.8 ± 1.3	34 ± 4.4	311 ± 52	0

TABLE 15-B
(Experimental arrangement No. 19)
Effect of COLA (ascending doses) on intact female rats in which hormone treatment was initiated 48 hours after preparation of the granuloma-pouch.

Group	*Treatment* COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	100	175 ± 2.4	+ 9.4	12.0 ± 0.6	245 ± 21.7	1007 ± 148.2	0
II	400	165 ± 2.2	+ 3.1	12.0 ± 1.9	140 ± 7.6	808 ± 79.7	0
III	1000	147 ± 2.6	— 8.1	6.4 ± 1.1	49 ± 6.3	488 ± 34.3	0
IV	5000	140 ± 2.4	— 12.5	6.0 ± 1.8	40 ± 1.7	392 ± 36.8	0

hormone treatment was initiated on the day on which the granuloma-pouch was prepared (Table 15-C).

The results were virtually the same as those obtained under standard conditions (cf. Table 15-B), that is when the hormone-treatment was initiated 48 hours after preparation of the granuloma-pouch.

In view of the previously mentioned great sensitivity of males, the same experiment was then repeated on *adrenalectomized, but not gonadectomized male rats* (Table 15-D).

Here, considerable mortality was noted at the daily dose-levels of 50 and 100 μg., but otherwise the response was essentially the same as in adrenalectomized females (8).

(20) *COLA* (*ascending doses*) + *STH* (*medium dose*).—In intact rats (1, 8) as well as in adrenalectomized animals maintained on NaCl (4), those actions of cortisol which are here under investigation can readily be inhibited by STH. On the other hand, preliminary experiments under slightly different conditions indicated that if adrenalectomized rats are maintained only on tap water STH cannot antagonize these cortisol actions (4). It was considered worthwhile to verify this latter point under the conditions of the present investigation since it is naturally of great importance to establish the indispensability of sodium or mineralocorticoids as necessary conditioning factors for the efficacy of STH. Hence, an experiment of this type was performed under standard conditions on adrenalectomized and ovariectomized rats maintained on tap water. Here again, one additional untreated control group was added for comparative purposes (Table 16).

Perusal of our data indicates that even 600 μg/day of STH failed to antagonize the effects of COLA significantly, at any dose level, in the absence of mineralocorticoids or salt supplements. This agrees well with the results of Experimental Arrangement No. 7, in which both these hormones were given in ascending doses (cf. Table 7). Indeed, the latter experiment had shown that even as much as 2000 μg/day of STH is ineffective in this respect.

(21) *COLA* (*ascending doses*) + *DOCA* (*medium dose*).—We had previously shown that, in adrenalectomized rats given ascending doses of cortisol and a medium dose of DOCA, the effects of the latter predominate at low and those of the former at high dose-levels. This fact was explained by the "law of intersecting dose-effect curves" (8).

(22) *COLA* (*ascending doses*) + *STH* (*medium dose*) + *DOCA*

TABLE 15-C
(Experimental arrangement No. 19)
Effect of COLA (ascending doses) on intact female rats in which hormone treatment was initiated on the day on which the granuloma-pouch was prepared.

Group	*Treatment* COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	100	168 ± 2.4	+ 5.0	17.0 ± 1.6	204 ± 15.2	860 ± 57.5	0
II	400	165 ± 2.2	+ 3.1	12.0 ± 2.1	202 ± 13.8	760 ± 54.9	0
III	1000	151 ± 3.6	— 5.0	5.0 ± 1.6	55 ± 2.7	476 ± 37.8	0
IV	5000	134 ± 2.2	— 16.2	1.3 ± 0.8	33 ± 2.7	304 ± 15.3	0

TABLE 15-D
(Experimental arrangement No. 19)
Effect of COLA (ascending doses) on adrenalectomized but not gonadectomized male rats.

Group	*Treatment* COLA (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	152 —	— 7.3	6 —	300 —	510 —	80
II	100	131 ± 7.2	— 18.6	7 ± 0.9	240 ± 21.4	483 ± 46.5	30
III	400	131 ± 4.6	— 20.6	4 ± 0.5	68 ± 5.2	457 ± 16.0	0
IV	1000	144 ± 4.5	— 10.6	5 ± 1.6	42 ± 3.6	432 ± 31.3	0

(*medium dose*).—This experiment was performed under standard conditions on adrenalectomized and ovariectomized rats (Table 17).

In comparing the results of this series with those of Experimental Arrangement No. 20 (cf. Table 16), the enormous importance of mineralocorticoid as a conditioning factor for STH actions and as an antagonist of COLA is particularly evident. Up to as high a dose as 400 μg/day of COLA, the catabolic, antiphlogistic and lympholytic effects of this glucocorticoid are considerably inhibited by combined treatment with STH and DOCA. At the dose of 2000 μg/day of COLA, the actions of the latter, and particularly its lympholytic effect, could not be fully counteracted by its antagonists.

(23) *COLA* (*ascending doses*) + *STH* (*ascending doses*);

(24) *COLA* (*ascending doses*) + *STH* (*ascending doses*) + *DOCA* (*medium dose*);

(25) *COLA* (*ascending doses*) + *DOCA* (*ascending doses*);

(26) *COLA* (*ascending doses*) + *STH* (*medium dose*) + *DOCA* (*ascending doses*);

(27) *COLA* (*ascending doses*) + *STH* (*ascending doses*) + *DOCA* (*ascending doses*).—These experiments correspond to the arrangements already discussed under Nos. 7, 8, 16, 17 and 9 respectively and hence they require no further discussion here.

Summary

This communication deals with the interactions between somatotrophic hormone (STH), cortisol acetate (COLA and desoxycorticosterone acetate (DOCA), as regards their effects upon somatic growth, inflammation and lymphatic-tissue development.

To evaluate these interactions it was necessary not only to determine the effect of each of these hormones alone and in combination, but also that of varying the dose-level of each substance, in animals receiving treatment with one or more hormones. This involved 27 integrated experiments under rigorously comparable conditions and necessitated the use of many more than the 2000 rats which were employed for the final tests described here in detail. Because of the large number of observations made, it would be impossible to comment on each point in this summary and hence only the general experimental arrangement and the principal findings will be summarized here.

In most instances, the test animals were simultaneously ovariecto-

TABLE 16
(Experimental arrangement No. 20)
Effect of COLA (ascending doses) + STH (medium dose) on adrenalectomized and ovariectomized rats.

Group	*Treatment* COLA (μg.)	STH (μg.)	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	—*	—*	142 ± 7.2	— 8.4	5.4 ± 1.4	287 ± 28.4	538 ± 76.8	50
II	50	600	140 ± 6.8	— 11.4	5.8 ± 1.8	265 ± 43.6	562 ± 65.3	10
III	100	600	148 ± 2.6	— 6.0	6.5 ± 0.4	260 ± 15.6	605 ± 93.1	0
IV	400	600	156 ± 2.2	— 1.1	5.8 ± 9.5	187 ± 10.5	549 ± 34.6	0
V	2000	600	152 ± 3.8	— 3.8	6.0 ± 1.4	51 ± 5.2	333 ± 27.2	0

* Adrenalectomized and ovariectomized, untreated controls.

TABLE 17
(Experimental arrangement No. 22)
Effects of COLA (ascending doses) + STH (medium dose) + DOCA (medium dose) on adrenalectomized and ovariectomized rats.

Group	*Treatment* COLA	STH	DOCA	Final Body-Weight (gm.)	Weight—Gain (%)	Exudate (ml.)	Thymus (mg.)	Spleen (mg.)	Mortality (%)
I	50	600	100	189 ± 2.7	+ 16.6	20 ± 1.9	470 ± 25.8	1146 ± 56.2	0
II	100	600	100	187 ± 4.6	+ 16.1	14 ± 2.1	526 ± 37.8	938 ± 53.5	10
III	400	600	100	182 ± 3.9	+ 13.0	11 ± 2.1	324 ± 15.3	812 ± 57.3	0
IV	2000	600	100	153 ± 3.1	— 4.9	5 ± 1.5	52 ± 5.3	346 ± 22.6	0

mized and adrenalectomized in order to deprive them of both their steroid-producing glands. In some instances, however, complementary experiments were performed on intact or merely adrenalectomized rats for control purposes.

The animals were kept under observation for 12 days. At the end of this period, their final body-weight acted as an indicator of somatic growth, the volume of exudate accumulating in the "granuloma-pouch" served as a measure of inflammation and the weight of the thymus and spleen helped to assess the development of the thymico-lymphatic organs.

Our principal findings may be summarized as follows:

1) In the adrenalectomized-ovariectomized rat, DOCA inhibits the catabolic, antiphlogistic and lympholytic effects of low doses of COLA. With increasing doses of DOCA, its "anticortisol actions" rise rapidly up to about 100 μg/day and then reach a plateau over which a further increase in effect cannot be obtained by raising the dose; the actions of COLA, on the other hand, do not rise as steeply with an increase in dosage, but continue to become more and more pronounced until near-fatal doses are reached. Consequently, at low dose-levels the former, at high dose-levels the latter, of these opposing hormone-effects will predominate. This is in agreement with the "law of intersecting dose-effect curves."

2) In intact rats, the anticortisol effects of DOCA are not manifest, presumably because the animals' own adrenals are already producing an optimal amount of mineralocorticoid (the equivalent of about 100 μg/day of DOCA).

3) The growth-promoting, prophlogistic and lymphatic-tissue stimulating effects of STH do not manifest themselves in adrenalectomized-ovariectomized rats unless the animals are given maintenance doses of DOCA or adequate dietary sodium chloride supplements. In this respect, DOCA is much more effective in conditioning the organism for the above STH effects than the substitution of 1% NaCl as a drinking fluid. COLA alone—in the absence of mineralocorticoid or of sodium chloride treatment—does not suffice to restore STH-responsiveness in such tests.

4) In none of the short-term experiments described in this communication was it possible, with the aid of STH (up to the dose-level of 5000 μg/day, to stimulate exudation or the development of the thymus and spleen above the maximal normal levels. This was so

irrespective of the presence or absence of the adrenals and of the type of corticoid therapy simultaneously given.

5) Among the various target-organs studied, the effect of COLA upon the thymus proved to be most resistant to inhibition by conjoint treatment with DOCA and/or STH.

6) The growth-promoting effect of STH, in adrenalectomized-ovariectomized rats given maintenance doses of DOCA, is intensely inhibited by COLA, even if the latter hormone is administered only at the minimum dose-level necessary for the maintenance of well-being. It follows that the growth-promoting effect of STH cannot manifest itself in the presence of normal amounts of glucocorticoids, such as are physiologically secreted by the adrenal cortex.

The authors are indebted to Pfizer Laboratories for "Cortril Acetate," to Schering Corporation for "Cortate" and to Armour Laboratories for "Somatotropin."—They are also grateful to Mr. W. Vins for statistical calculations.

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Growth, 1955, **19**, 137-140.

BOOK REVIEWS

An Introduction to Cell and Tissue Culture. The Staff of the Tissue Culture Course, Cooperstown, New York. 1949-1953. 123 pp. $4.00. Burgess Publishing Co.

This is an outline of the work of the Tissue Culture course given at Cooperstown, New York, 1949-1953. It is sponsored by the National Cancer Institute, the United States Public Health Service, The Tissue Culture Association and the Mary Imogene Bassett Hospital where the course was given. The resident staff consisted of Doctors Hank, Fawcett, Scherer, Leighton and Porter. The visiting staff included Doctors Carpenter and Earle. All have contributed to the volume.

The course developed from a long felt need for persons trained in tissue culture technique who would be able to carry on investigations in many different fields of research. It is planned so that tissue culture work can be carried on with simple equipment such as that found in almost any laboratory.

The first part of the book contains suggestions about equipment and supplies to be used and the preparation of various solutions. Then follows different methods of growing tissues; on slides, in test tubes, in flasks and on sponges. The cultivation of tissue cells for virus propagation is fully discussed.

Part II is a review of the lectures given in the course. They cover cell types, patterns of growth, cell differentiation, dedifferentiation and cell modulation. The various nutrient media such as serums, embryonic extracts and unincubated eggs are discussed with an evaluation of some of the synthetic media. There is a short chapter on organ culture.

Part III contains discussions on special applications of tissue cultures to virology and the study and diagnosis of cancer tissues.

The editor asks for suggestions. The book would be easier to read if there were two columns instead of one to a page or if smaller pages were used. It is difficult to follow the long lines across the page in the type used.

The book is an excellent outline of a course on the growth of tissues *in vitro* and their uses in solving the many problems arising in the various fields of biological research. Each chapter has a well chosen bibliography.

M. J. HOGUE

Brookhaven Symposia in Biology No. 6, Abnormal and Pathological Plant Growth. Brookhaven National Laboratory, Upton, N.Y. August, 1953. $2.10. 303 pages.

The Brookhaven Symposium on Abnormal and Pathological Plant Growth was both a timely and stimulating one. Timely because of the possible implications of the subject to human cancer, and stimulating because cancer research is fast reaching a point—similar to the recent history of polio research—where one inroad or another of the various avenues of approach will with a fair degree of certainty lead to the ultimately sought objective of cure and prevention of cancer.

One might have wished for a more direct approach to a correlation between normal growth and the types discussed. Actually—and no doubt intentionally—some of this is included (papers by Skoog, Wetmore, LaRue, possibly the Allens). It is almost certain that the correct preventive to the abnormal is through an understanding and correlation of the normal systems. And likely because of this logical point of view, the scope of the subject matter was exceedingly and advantageously broad, covering the analysis of normal patterns of growth and "abnormal" growths due to chemical, physical, biological, etiological, and viral causes or factors. In order to get a broad view of the total problem one should read Block's fine overall scope of the subject. Except that the first two papers deal with the more "normal" pattern of growth, all others can be placed under one or the other groupings which Block so conveniently outlines.

However, there appears to be one glaring omission under Block's section on *Transformation-Tumors* (p. 45). This is probably not intentionally so, since the author (Block) realized that it was to appear in a subsequent chapter as a contribution to the symposium. The reference is to the exciting and intriguing findings of Klein on the proposed mechanism of crown-gall induction. The latter paper is probably the most stimulating of the biochemical approaches to the problem of abnormal and pathological growth. And certainly one is willing to admit that in 9 chances out of 10, the problem has basically a biochemical and physiological origin; and consequently the solution is likely to come from this same approach ultimately. Klein's paper (abnormal) along with Skoog's (normal) seem to have presented a somewhat challenging and fresh point of view. The discussions of these two papers alone indicate this (these two papers received the most discussion).

Braun reviews a rather broad view of the problem morphologically, genetically, and physically (temperature-time relationship) on the origin (induction) of the tumor principle. In basic intent it is similar to Klein's but from altogether another approach. Some of the results and conclusions of teratomal studies would seem to be subject to certain of the points raised in Kehr and Smith's paper on genetic tumors, namely, that one has to be extremely careful that the cells or tissue with which one is working are homozygously pure (a point challenged by White in both papers). Equally interesting discussion evolved from this point.

Ending up the biochemical-physiological papers was one by de Ropp who presents a short, but presently attractive and popular point of view in cancer therapy. His hypothesis is one based on the ideas expressed previously by Went, Thimann, Skoog, et al. concerning correlation and physiological balance of biochemical reactions and responses. A fresh point of view involving the concepts of Tang and Bonner and Galston, et al. on IAA-oxidase is presented. Also, data are presented pertaining to the screening and use of several agents used in chemotherapy and animal cancer (e.g. streptomycine, antifolics, 8-azaguanine, etc.). Nickell's paper on virus-tumor growth goes into the use of purines, their derivatives and analogs, and antibiotics more fully than de Ropp's.

The last six papers (excluding Nickell's) deal primarily with morphological-etiological factors on "abnormal" growth responses. Three of these deal with viral infections, one on phylogenetic regeneration and morphogenesis, one on bacterial symbiosis (root nodule), one on insect gall (Solidago gall), and one on growth caused from ionizing irradiations.

For the student of phylogeny and "abnormal normalities" (or vice versa?), LaRue's paper presents an interesting point of view. From observations on the growth of gymnosperm gametophytes LaRue is able to conclude that in unnatural conditions (i.e. growing tissues *in vitro*) many results can be obtained which may never have occurred in nature or in the ancestry of these organs. This raises doubt concerning the much quoted maxim that "ontogeny recapitulates phylogeny."

The final paper by Gunckel and Sparrow shows some vivid examples of the effects of irradiation on various organs of several species. The authors are emphatic in two points: (1) most morphogenetic

abnormalities are basically physiological or biochemical in nature, and (2) most of the irradiation effects are quantitatively rather than qualitatively different from those known to occur naturally.

The close to 300-page, 15-paper compilation can be highly recommended for people working in the field of abnormal growth, including cancer research, both plant and animal. One of the possible deterrent factors of the symposium is the fact that it had in attendance almost exclusively the people who were directly in the field (plant) and very few members from "across the track" in the animal pathology or cancer research field. This should be remedied in future symposia of this type. Something of this kind was attempted at the recent Tissue Culture Association meeting in Philadelphia. More and more the problems of basic physiology and biochemistry are the same for plant and animal cells, for normal cells and possibly equally so for the abnormal growth. Thus a joint understanding on these common-ground things may bring about the more rapid solution to the specific problems of each separately in these two allied areas of biology.

JAMES H. M. HENDERSON